Volume 21 Number 6

JOURNAL
of
DENTAL RESEARCH

FOUNDED BY WILLIAM J. GIES IN 1919

Official Publication of the

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

December, 1942

Dental Prosthetic Restorations Constructed for Naval Personnel. *C. C. DeFord* and *C. A. Schlack* (U. S. Naval Dental School) 493

The Effect of Strontium on the Incisor of the Rat. I. Injection of Small Doses of Strontium Chloride as a Means of Measuring the Rate of Incremental Dentin Apposition. *J. P. Weinmann* (Chicago College of Dental Surgery) 497

Effect of Saliva on Capillary Permeability. *B. M. Levy* and *J. L. T. Appleton* (University of Pennsylvania) 505

Opsonin in the Saliva of Caries-Negative and Caries-Positive Patients. *C. W. Hammond* and *J. P. Weinmann* (Chicago College of Dental Surgery) 509

The Effect of a Low Calcium and Vitamin D Free Diet on the Skeleton and Teeth of Adult Rats. *A. P. Lund* and *W. D. Armstrong* (University of Minnesota) 513

Histochemical Study of Phosphatase and Glycogen in Fetal Heads. *N. H. Horowitz* (University of Southern California and California Institute of Technology) 519

Ameloblastoma: Review of 34 Cases. *J. L. Bernier* (Army Medical Museum) 529

Cultivation and Isolation of Mouth Spirochetes. *O. W. Wichelhausen* and *R. H. Wichelhausen* (Johns Hopkins University) 543

Relationship of the Gingival Crevice and the Alveolar Crest. *H. M. Goldman* (Harvard University) 561

Supplement.

I. Sustaining Subscribers to the Journal 565

II. International Association for Dental Research. Register of Members as of December 1, 1942. Compiled by E. H. Hatton 566

III. Books Received, 1942 578

Index to Volume 21 579

General Matter for Volume 21:

Title Pages i

Contents of Volume iii

PUBLISHED BI-MONTHLY AT MT. ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.
By THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORIAL OFFICE: 4559 Scott Ave., St. Louis, Mo.

Copyright, 1942, by the International Association for Dental Research

Made in United States of America

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORS OF THE JOURNAL OF DENTAL RESEARCH

Editor Emeritus—William J. Gies

Publication Committee: J. Frank Hall, S. S. Arnim and Hamilton B. G. Robinson.

Editor—Hamilton B. G. Robinson. *Business Manager*—J. Frank Hall. *Circulation Manager*—S. S. Arnim.

Assistants to the editor—L. R. Boling and W. E. Koch, Jr. *Assistant to the business manager*—R. L. Simpson, Jr. *Assistant circulation manager*—Virgil D. Cheyne.

Board of Editors—Editor-emeritus, members of the Publication Committee and sectional representatives named in fifth column below.

THE SECTIONS—THEIR SECRETARIES, AND REPRESENTATIVES IN THE COUNCIL AND IN THE BOARD OF EDITORS (1941)

Division	*Section*	*Secretary*	*Councillor*	*Editor*
Canada	Halifax	S. G. Ritchie	J. S. Bagnall	S. G. Ritchie
	Toronto	A. W. Ellis	A. D. Mason	Thos. Cowling
	Winnipeg	E. Roy Bier	H. J. Merkeley	E. Roy Bier
China	Chengtu	R. Gordon Agnew	R. Gordon Agnew	R. Gordon Agnew
England	London	Evelyn Sprawson	Evelyn Sprawson	Evelyn Sprawson
Germany	Prague	Friedr. Neumann	Friedr. Neumann	Jan Jesensky
	Vienna	Fritz Driak	R. Trauner	F. Shönbauer
Hungary	Budapest	Ferenc Köszeg	———	———
Palestine	Palestine	S. Lewin-Epstein	S. Lewin-Epstein	S. Lewin-Epstein
South Africa	Johannesburg	Julius Staz	J. N. Noriskin	W. B. Osborn
United States	Ann Arbor	Philip Jay	Philip Jay	Philip Jay
	Baltimore	M. S. Aisenberg	M. S. Aisenberg	———
	Boston	G. E. Thompson	L. M. S. Miner	P. E. Boyle
	Chicago	M. K. Hine	A. G. Brodie	L. S. Fosdick
	Cleveland	S. W. Chase	Thomas J. Hill	S. W. Chase
	Columbus	R. D. McFarland	J. H. Kaiser	H. D. Spangenberg
	Detroit	Samuel J. Lewis	P. C. Lowery	Samuel J. Lewis
	Houston	E. Kotanyi	E. Kotanyi	E. Kotanyi
	Indianapolis	Hannah E. Silberstein	V. D. Cheyne	T. D. Speidel
	Iowa City	A. O. Klaffenbach	A. W. Bryan	George S. Easton
	Louisville	Raymond E. Myers	J. T. O'Rourke	H. T. Knighton
	Minnesota	W. J. Simon	P. J. Brekhus	W. D. Armstrong
	New Orleans	C. S. Tuller	S. L. Tiblier	F. J. Wolfe
	New York	Isaac Neuwirth	L. M. Waugh	Frances Krasnow
	Philadelphia	Thomas J. Cook	J. L. T. Appleton	J. L. T. Appleton
	Pittsburgh	L. E. Van Kirk	H. E. Friesell	L. E. Van Kirk
	Richmond	A. P. Little	A. D. Brashear	Harry Lyons
	Rochester	H. B. McCauley	H. B. McCauley	H. B. McCauley
	San Francisco	A. J. Ker	W. C. Fleming	Herman Becks
	St. Louis	R. C. Wheeler	W. E. Koch, Jr.	R. F. Bleiker
	Washington	H. W. Krogh	F. S. Arnold	C. A. Schlack
Materials Group		E. W. Skinner	W. S. Crowell	I. C. Schoonover

OFFICERS OF THE ASSOCIATION

President—Charles F. Bodecker, *President-elect*—Philip Jay, *Vice-president*—H. Trendley Dean, *Secretary-treasurer*—Edward H. Hatton, *Editor*—Hamilton B. G. Robinson, *Trustees*—R. W. Bunting, Edward H. Hatton, Thomas J. Hill, Arthur H. Merritt, Leuman M. Waugh.

Subscription price $5.00 per volume. Single numbers when available $1.00.

Entered as second-class matter June 24, 1919, at the Post Office at Baltimore, Maryland, under the act of March 8, 1879

WAVERLY PRESS, INC

BALTIMORE, U. S. A.

DENTAL PROSTHETIC RESTORATIONS CONSTRUCTED FOR NAVAL PERSONNEL[1]

C. C. DE FORD[2] AND CARL AUGUST SCHLACK[2]

U. S. Naval Dental School, Bethesda, Md.

A review of the dental literature here available does not disclose detailed quantitive data on type prosthetic restorations actually constructed in private practice or in clinics at various institutions. Gross reports are published periodically by the Armed Forces but these do not include denture classification or frequency of construction of any type. Because such information may facilitate more accurate budget estimates for certain dental materials as well as to support the necessity of emphasis on "man-hour" requirements for rendering such dental treatment, an investigation of this nature was chosen.

PURPOSE

This study was made principally to acquire more detailed quantitive data on type prosthetic restorations actually constructed and worn by patients. This investigation is not concerned with materials used nor with the design of each case.

PROCEDURE

Data was obtained from the files on dental prosthesis at the U. S. Naval Dental School. These covered appliances constructed at the school in the years 1938, 1939, and 1940. In all, 587 patients received denture service with a total of 891 dental prosthetic appliances constructed.

The appliances were classified to show the following combinations (Table I and *fig. 1*): 1—Complete maxillary and mandibular denture cases combined. 2—Complete maxillary denture cases only. 3—Complete mandibular denture cases only. 4—Complete maxillary and partial mandibular denture cases combined. 5—Complete mandibular and partial maxillary denture cases combined. 6—Partial maxillary and partial mandibular denture cases combined. 7—Partial maxillary denture cases only. 8—Partial mandibular denture cases only.

Another classification (Table II and *fig. 2*) was made to show the number of partial appliances that were constructed to replace specific numbers of missing teeth.

RESULTS

1. The tables and graphs indicate that, in the years 1938 to 1940 inclusive, partial maxillary and partial mandibular denture combinations were most fre-

[1] Received for publication July 29, 1942.

[2] Lieutenant Commander, D. C., U.S.N.

TABLE I

Prosthetic Denture Combinations

COMBINATIONS	NUMBER OF PATIENTS	PROSTHETIC APPLIANCES	NO.
Complete maxillary & full mandibular	78	Complete maxillary	170
Complete maxillary only	21	Complete mandibular	85
Complete mandibular only	5	Partial maxillary	298
Complete maxillary & partial mandibular	71	Partial mandibular	338
Complete mandibular & partial maxillary	2		
Partial maxillary and partial mandibular	153		
Partial maxillary only	143		
Partial mandibular only	114		
Total	587	Total	891

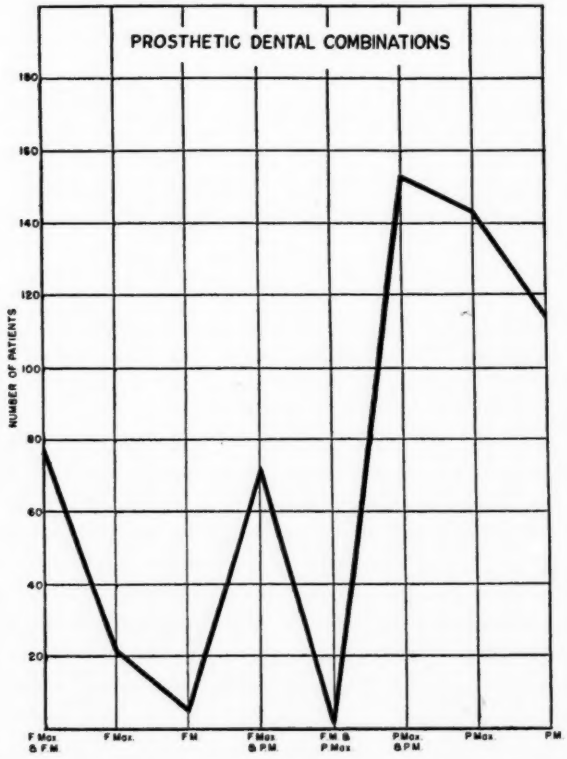


FIG. 1. Prosthetic denture combinations. F. Max.—complete maxillary, F. M.—complete mandibular, P. Max—partial maxillary, P.M.—partial mandibular.

quently constructed (26.6%) and complete mandibular and partial maxillary denture combinations most infrequently (0.85%). 2. Of the partial maxillary dentures constructed those supplying 5 teeth were most frequent (19.1%) and

TABLE II

Partial Dentures Supplying Specific Numbers of Teeth

NUMBER OF TEETH	NUMBER OF MAXILLARY APPLIANCES	NUMBER OF MANDIBULAR APPLIANCES
1	15	13
2	24	21
3	46	51
4	50	67
5	57	66
6	37	45
7	30	24
8	20	29
9	12	10
10	5	7
11	2	4
12	0	1
Total.....	298	338

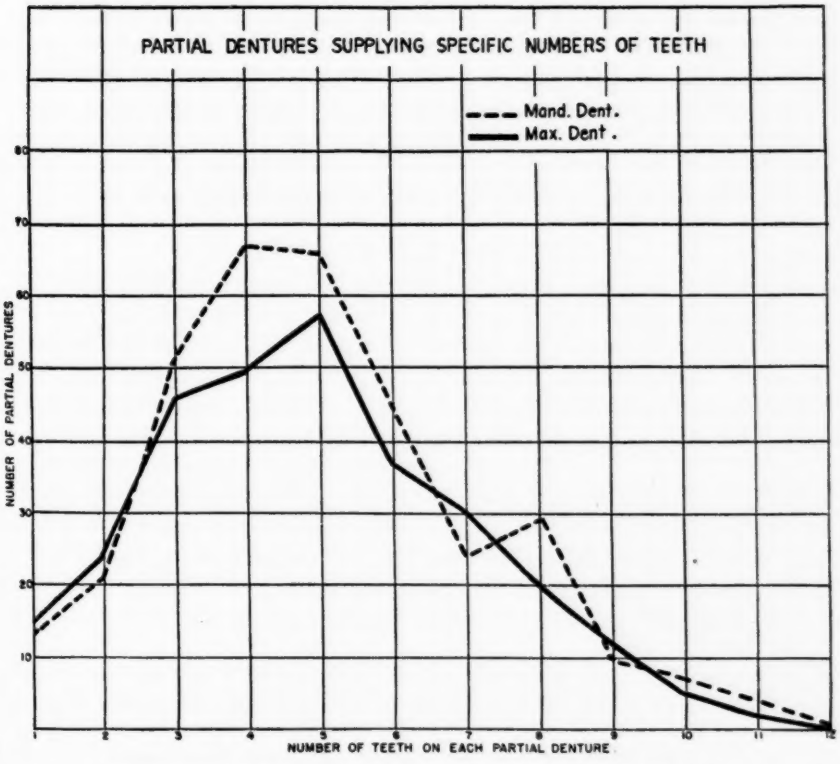

PARTIAL DENTURES SUPPLYING SPECIFIC NUMBERS OF TEETH
Mand. Dent.
Max. Dent.
NUMBER OF PARTIAL DENTURES
NUMBER OF TEETH ON EACH PARTIAL DENTURE

FIG. 2. Partial Dentures Supplying Specific Number of Teeth

partial maxillary dentures supplying 11 teeth were rarely made (0.7%). 3. Of the partial mandibular dentures constructed those supplying 4 teeth were most frequently constructed (19.8%) and those supplying 12 teeth most infrequently (0.3%). 4. 28.6% of all dentures constructed were complete cases and 71.3% were partial. 5. Complete mandibular dentures comprised 9.5% of all dentures constructed, complete maxillary 19.1%, partial maxillary 33.4% and partial mandibular 37.9%.

CONCLUSION

From data obtained at the U. S. Naval Dental School it appears that prosthetic dental replacements most necessary for naval personnel here treated are of the partial denture type.

The opinions or assertions contained in this article are the private opinions or assertions of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.—Article 113, U. S. Navy Regulations.

THE EFFECT OF STRONTIUM ON THE INCISOR OF THE RAT[1]

I. Injections of Small Doses of Strontium Chloride as a Means of Measuring the Rate of Incremental Dentin Apposition

JOSEPH P. WEINMANN, M.D.

Foundation for Dental Research, Chicago College of Dental Surgery, Chicago, Ill.

INTRODUCTION

Strontium has chemical properties similar to calcium. This is the reason that its effect on bony structures has been studied frequently during the last half century (13, 9, 15, 10, 11, 12, 22, 23, 21, 14). Many pharmacological studies of strontium as a substitute for calcium have been made (4, 1, 3, 2) but few references to its effect on the teeth are available (6, 8). The effect of strontium on the structure of teeth has not been investigated histologically.

In a series of experiments 2 methods for studying the effect of strontium chloride on the dental tissues of the rat incisor were used, 1, injecting single and multiple doses of strontium chloride and, 2, feeding varying amounts of the chemical. This report deals with the results obtained by the injection of small amounts of strontium chloride. It was found that by injecting small amounts, changes in dentin occur which permit the measuring of the rate of dentin apposition just as when alizarin red S or sodium fluoride are injected (16, 19).

MATERIAL AND METHODS

The present report is based on the study of the upper incisors of 23 rats varying in age from 23 to 96 days. In the first group of experiments 6 animals were injected with 1 cc. of a 2.75 per cent strontium chloride solution from 1 to 34 times. Five controls received 1 cc. of physiologic saline solution. These experiments are recorded in Table I. In a second group of experiments rats from 3 different litters were injected with strontium chloride and alizarin red S. The data for these experiments are compiled in Table II.

Albino rats bred from Wistar Institute stock were fed Dog Chow (Purina). This diet was augmented twice a week by the addition of lettuce, carrots and dry bread crusts. The animals were kept in a light room, 3 to 5 in a cage. The animals were weighed at the beginning and the end of the experiments; if the experiments extended over a week or more, the animals were weighed once a week.

Strontium chloride, purified (J. T. Baker Chemical Co., Phillipsburg, N. J.) was dissolved in distilled water in concentrations of 0.7, 1.4 and 2.75 grams

[1] Read at a meeting of the Chicago Section of the International Association for Dental Research, Oct. 9, 1942. Received for publication June 15, 1942.

TABLE I

Daily weight gains and rate of apposition of dentin of upper incisor of 6 rats injected 1 to 34 times with 1 cc. 2.75% $SrCl_2$ and from 5 controls injected with physiologic saline solution

RAT NO.	SEX	AGE AT START	WT. AT START	WT. AT END	% $SrCl_2$ OR NaCl 1 cc. INJ.	$SrCl_2$ IN MG./ KILO. BODY WEIGHT	NO. INJ.	LENGTH EXP.	DAILY WT. GAIN	DAILY RATE DENTIN APPOS.
		days	*grams*	*gram*				*days*	*grams*	*μ*
20	♂	34	90.9	?	2.75%	300	1	2	?	18
22	♀	34	81.9	90.5	2.75%	340	2	7	1.2	20.6
27	♂	35	56.9	89.0	2.75%	450	3	10	3.2	18.9
33	♀	35	66.9	127.0	2.75%	390	8	28	2.1	*
35	♀	23	40.9	283.0	2.75%	680 to 100	30	114	2.1	16.0
38	♂	23	35.9	377.0	2.75%	680 to 70	34	140	2.4	*
21	♀	34	85.9	89.5	NaCl		2	7	0.5	
26	♂	35	66.9	110.0	NaCl		3	10	4.3	
32	♀	35	59.4	130.0	NaCl		8	28	2.5	
34	♀	23	26.9	220.0	NaCl		30	114	1.7	
40	♀	23	32.9	238.0	NaCl		34	140	1.4	

* The daily rate of apposition was not obtained because ground sections were not available.

TABLE II

Comparison of daily weight gains and rate of apposition of dentin of upper incisor after injection of $SrCl_2$ and alizarin in varying concentrations

Explanation of abbreviations: 0.3A and 1.0A, 0.3 and 1.0 cc. respectively 2% alizarin red S; 0.7S, 1.4S and 2.75S, 1.0 cc. respectively of 0.7%, 1.4% and 2.75% Strontium chloride.

RAT NO.	SEX	AGE AT START	WEIGHT IN GRAMS AT:			INJECTION				$SrCl_2$ MG./ KILO. BODY WEIGHT	ALIZARIN MG./ KILO. BODY WEIGHT	TIME OF EXP.	DAILY WT. GAIN	DAILY RATE OF DENTIN APPOS. AFTER INJ. OF:	
			1st inj.	3rd inj.	End of exp.	1st	2nd	3rd	4th					Aliz	$SrCl_2$
		days										*days*	*grams*	*μ*	*μ*
262	♂	36	62.3		65	0.3A	0.7S	0.7S		110	100	6	0.4	17.5	18
263	♂	36	61.3		61	0.3A	0.7S	0.7S		110	100	6	−0.1	19	19.7
259	♂	36	65.5		70.5	0.3A	1.4S	1.4S		220	100	6	0.8	18.7	18.7
260	♂	36	59.4		66.4	0.3A	1.4S	1.4S		220	100	6	1.1	21	21
264	♀	36	60.3		62.5	0.3A	2.75S	2.75S		450	100	6	0.4	16.5	17.5
267	♀	36	56.3		57.2	0.3A	2.75S	2.75S		450	100	6	0.1	19.5	19.5
102	♂	94	294		291	2.75S	1.0A	2.75S		90	60	7	−0.4	16	15
121	♀	92	280		248*	2.75S	1.0A	2.75S	2.75S	90	70	9		15	17
115	♂	96	263	254	255	1.0A	2.75S	2.75S		100	70	15	−0.5	16.2	19.3
508	♂	73	141.5	132.4	161.2	1.0A	1.0A	2.75S	2.75S	200	140	15	1.3	9.5	20
510	♀	73	118.2	125.1	121.1	2.75S	2.75S	1.0A	1.0A	200	160	15	0.2	12	19
511	♀	73	133	141.2	138	2.75S	2.75S	1.0A	1.0A	190	140	15	0.3	15.0	20.0

* Gave birth to litter (6) on day of last injection.

per cent. The 2.75 per cent strontium chloride solution is an isotonic solution having a pH of 5.2. The injections were given subcutaneously and under aseptic conditions. Alizarin red S in 2 per cent solution was administered according to

the method described by Schour, Hoffman, Sarnat and Engel (16). The animals were killed by prolonged anaesthesia with chloroform. The specimens were immediately fixed in Zenker-Formol (Maximow) for 1 day or in Bouin's solution for 2 days. One-half of the upper and lower jaws were decalcified in 5 per cent nitric acid, embedded in celloidin, stained with hematoxylin-eosin, Mallory or impregnated with silver. From the upper incisors of the other half of the jaws, ground sections[2] were prepared and investigated unstained.

FINDINGS

A. *General Effect*—Subcutaneous injections of 1 cc. of strontium chloride in doses varying from 0.7 to 2.75 per cent produced no local irritation which could be observed clinically. The animals injected 1 or more times with isotonic strontium chloride solution gained well in weight compared with control animals of the same litter (Table I). The daily gain in weight of the experimental animals varied from 1.2 to 3.2 gms., the gain of the control animals from 0.5 to 4.3 gms.

Intraperitoneal injections of 2 per cent alizarin S caused a greater weight loss in older rats than in younger. In the cases where strontium was administered before alizarin the animals gained weight after the injection of strontium but lost weight after the injection of alizarin (Table II).

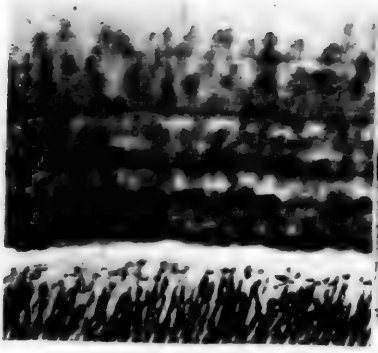
B. *Effect on Enamel*—The changes in the enamel organ after the injection of small doses of strontium chloride were limited to the ameloblasts and were of very slight degree. Elevation of the ameloblastic layer from the enamel matrix was seen occasionally. In a few instances a delicate incremental line could be found in both the decalcified and the ground sections.

C. *Effect on Dentin*—Regular and distinct marks in the dentin are produced by small doses of strontium, even after the injection of 70 mg. of strontium chloride per kilogram of body weight. They persist after multiple injections as well. In decalcified sections stained with hematoxylin-eosin an immeasurably thin line can be seen. This line is stained more intensely with hematoxylin than the dentin formed previous to the injection (*figs. 1* and *2*). The dentin bordering this line on the pulp side appears striated due to the alternate arrangement of paired light and dark zones. The effects are most distinct in the middle and the incisal portions of the tooth than in the basal and are more accentuated on the labial than on the lingual side. In unstained ground sections only one effect of the injections is clearly visible. An extremely delicate line can be observed which corresponds to each injection of strontium chloride solution (*figs. 3, 4* and *5*). This line is similar to the intensively stained one observed in decalcified sections.

D. *Rate of Dentin Apposition*—The distinct marking of the dentin by the injection of strontium chloride suggested the measurement of the rate of dentin

[2] We wish to thank Dr. Schour for the use of the equipment in his laboratory in preparing ground sections. We also wish to express our appreciation to Dr. M. Hoffman and Mr. W. J. Furuta for their help in this phase of the investigation.

FIG. 1



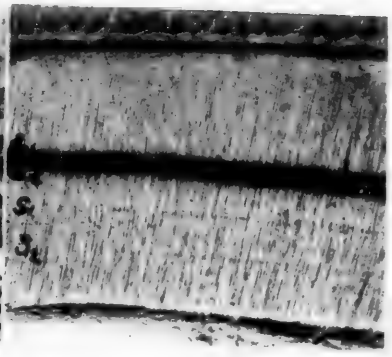


FIG. 4

FIG. 2

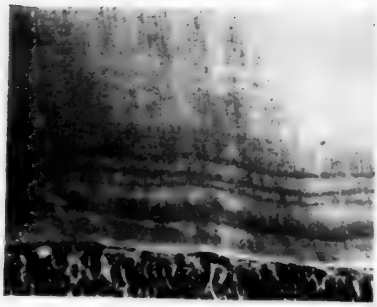


FIG. 5

FIG. 3

FIG. 1. Longitudinal decalcified section of upper rat incisor. R22 (Table I). Two injections of 1 cc. 2.75% $SrCl_2$ at interval of 3 days (S_1 and S_2). Four days after the second injection, sacrificed. Dentin formed previous to injection as well as afterwards shows globuli as sign of inadequate calcification. Between 2 strontium lines (S_1 and S_2) 2 pair of light and dark zones can be distinguished. Orig. mag. ×230.

FIG. 2. Longitudinal decalcified section of upper rat incisor. R263 (Table II). One injection of alizarin (a_1) and 2 of 1 cc. 0.7% $SrCl_2$ (S_1, S_2) at intervals of 2 days. Two days after last injection, sacrificed. Dentin formed before alizarin injection appears to be homogeneously calcified. Between alizarin injection and first strontium chloride injection dark stained strip (a_2). Strontium lines are followed by lighter-stained strip. Orig. mag. ×230.

apposition occurring between 2 consecutive injections. The method of measurement was the same Schour, Hoffman, Sarnat and Engel (16) employed following the injection of alizarin and sodium fluoride. The measurements reported here were made on the labial portion of ground and decalcified sections of the upper incisor.

The first experiments (Table I) showed the rate of dentin apposition to be slightly higher than would be expected from the figures reported following the injection of alizarin red S into young rats (7). In order to ascertain that the marks observed were caused by strontium and to compare the rate of dentin apposition in the same specimen, rats were injected with strontium chloride and with alizarin red S, the alizarin being injected either before, between or after the injections of strontium.

Six litter mates 5 weeks old were injected with a small dose of alizarin red S and 2 days later were given 2 injections of 1 cc. strontium chloride 48 hours apart in concentrations ranging from 0.7 to 2.7 per cent. Although the rate of dentin apposition showed a difference after alizarin and strontium injections, the numerical significance of which can be doubted, direct inspection of the microscopic sections suggested a higher rate after the strontium injection than after alizarin [*figs. 2* (R263) and *3*, (R262)]. Three animals from different litters, all 3 months old (Table II, R102, 121, 115), showed the rate of dentin apposition to vary according to figures previously reported (15). Two of 3 animals injected showed a greater rate of dentin apposition after strontium injections than after alizarin injections. One animal showed a reverse condition.

Three litter mates ranging in age and weight between the 2 previous groups received 2 alizarin and 2 strontium chloride injections (Table II). The amount of alizarin injected was above the dosage suggested as optimal (16). Between the 2 alizarin injections, respectively, and the 2 strontium injections there was an interval of 3 days, but between alizarin and strontium there was a 4 day interval (*figs. 4, 5* and *6*). In all instances the rate of dentin apposition was found to be significantly smaller after the injection of alizarin S than after injection of strontium. The rate was below the average after the injection of alizarin and above the average after the injection of strontium.

FIG. 3. Longitudinal ground section of upper rat incisor. R262 (Table II). a, alizarin line; S_1, S_2, strontium lines (1 cc. 0.7% $SrCl_2$). Injections were given at intervals of 2 days and 2 days after last injection animal was sacrificed. Orig. mag. ×160.

FIG. 4. Longitudinal ground section of upper rat incisor. R508 (Table II). Two alizarin injections at 3 day interval (a_1, a_2). After 4 days 2 strontium injections (1 cc. 2.75%) at interval of 3 days. Five days after last injection rat was sacrificed. E, enamel. Orig. mag. ×140.

FIG. 5. Transverse ground section of upper rat incisor R511 (Table II). Two strontium injections (S_1, S_2) at interval of 3 days. After 4 days 2 alizarin injections at interval of 3 days (a_1, a_2). Five days after last injection rat was sacrificed. E, enamel. Granulated appearance of the dentin formed after the second alizarin injection, d. U, union of the 2 alizarin lines.

DISCUSSION

Injections of strontium chloride proved to be a very feasible method for establishing a means for measuring the rate of dentin apposition even in concentrations of 70 mg. per kg. of body weight. It may be possible that even smaller doses will leave a mark in the forming dentin.

Previously 2 methods to measure the rate of dentin apposition have been recommended: the injection of alizarin and fluorine. All 3 methods, alizarin, sodium fluoride and strontium chloride, produce marks which permit the measuring of the increase in thickness or the incremental apposition of the dentin. The point must be stressed that these methods do not allow the measuring of the axial growth of the tooth or its elongation. All 3 methods of measuring the incremental apposition have in common the use of drugs which influence dentin formation or calcification.

Alizarin S marks the layer of dentin (and bone) which is calcifying, presumably, at the time of the injection (14). Schour and his co-workers demonstrated a temporary loss of weight after the injection of alizarin S and stated that young rats are more resistant to alizarin than old animals. These observations are confirmed by the present experiments. In addition, the present experiments demonstrate that alizarin in doses of 140 mg. per kg. of body weight produces disturbances in the dentin formation. *Fig. 5* shows a transverse ground section of an upper rat incisor. At intervals of 3 days this animal (#511) received 2 injections of strontium chloride and 2 injections of alizarin S in a concentration of 140 mg. per kg. of body weight. The strontium lines are found parallel to each other around the entire circumference of the tooth. The 2 alizarin lines, however, unite at the level of the cemento-enamel junction. Schour and coworkers (18, 5) observed that in vitamin A as well as in magnesium deficiency the cemento-enamel junctions are critical sites where the growth gradients change suddenly. *Fig. 5* demonstrates that such a pathologic change might occasionally be produced by alizarin S. In addition *fig. 5* shows that the dentin formed after the second alizarin injection has a granulated appearance, probably due to some disturbance in formation or calcification. *Fig. 2* shows that the alizarin injection produces changes in the forming dentin which can be observed in the decalcified section. The first line, a_1, occurred apparently at the time of injection and the second line, a_2, a day later. From these findings it can be concluded that alizarin in doses higher than 100 mg. per kg. of body weight may exert not only a systemic effect on the growth of the entire animal, but also a local effect on the formation and calcification of dentin. The low rate of incremental apposition observed after the injection of alizarin, especially in the older animals, as compared with that after the injection of strontium chloride might be explained, at least partially, by a general depressing effect of alizarin S. The retardation is impressive after the injection of alizarin in doses higher than those suggested as optimal (16).

Sodium fluoride produces lines in enamel and dentin which are formed at the time of its administration. The injuring effect of sodium flouride on dentin for-

mation was demonstrated by Schour and Smith (19). These authors found that the injection of sodium flouride produces a pair of layers, a light layer representing a trauma in calcification, and a dark layer representing a recovery reaction. Doses of sodium fluoride large enough to produce a fluorine line in the dentin cause disturbances in the enamel also. There are no data available concerning the influence of sodium fluoride injections on the growth curve of rats.

Strontium chloride solution in small doses produces changes which are almost entirely confined to the dentin. Each injection causes the appearance of a dark line which separates the dentin formed previous to the injection from the dentin deposited after the injection. This line, visible in ground as well as in decalcified, stained sections, serves as a convenient means for measuring the rate of dentin apposition. In decalcified, stained sections a striation is visible which is almost invisible in unstained ground sections. In contrast to alizarin, strontium chloride does not depress the growth curve of rats even after repeated injections of small doses. The larger rate of incremental apposition after injections of strontium chloride compared to that after alizarin injections shows that strontium in the amounts used in the present experiment has no depressing effect on dentin formation.

The drugs used as a means for measuring the rate of incremental apposition exert some toxic effect. The results of measuring the rate of incremental dentin apposition will be influenced by the drug and the concentrations used.

SUMMARY AND CONCLUSIONS

1. This study is based on observations made on 23 rats, 23 to 96 days old, which were given subcutaneous single or multiple injections of strontium chloride in concentrations of 70 to 680 mg. per kg. of body weight.

2. Subcutaneous injections of strontium chloride even in a concentration of 70 mg. per kg. of body weight produce sharp incremental lines in the dentin growing and calcifying at the time. These markings can be used to measure the rate of dentin apposition. The enamel shows a less sensitive reaction to strontium.

3. Single and multiple injections of strontium chloride do not retard the normal rate of increase in body weight.

4. Measurement of the rate of dentin apposition suggested a faster rate after injection of strontium chloride than after alizarin red S.

REFERENCES

1. Boriani, A., *Arch. di. farmal. sper.*, **67**: 119, 1939.
2. Campbell, W. W., and Greenberg, D. M., *Proc. Nat. Acad. Sc.*, **26**: 176, 1940.
3. Cole, V. V., Harned, B. K., and Hafkesbring, R., *J. Pharm. and Exper. Ther.*, **71**: 1, 1941.
4. Gerlach, W., and Miller, R., *Virchow Archiv*, **34**: 210, 1936.
5. Gagnon, J., Schour, I., and Patras, M. C., *Proc. Soc. Exper. Biol. & Med.*, **49**: 662, 1942.
6. Gies, W. J., *J. A. D. A.*, **5**: 527, 1918.
7. Herzberg, F., and Schour, I., *Anat. Record*, **80**: 497, 1941.
8. Klein, H., Becker, J. E., and McCollum, E. V., *J. D. Res.*, **10**: 733, 1930.

9. KOENIG, T., *Z. für Biol.*, **10:** 69, 1874.
10. KORSAKOW, N., *On the Problem of Pathogenesis of Rickets.* Thesis. (Moskau, 1883). Sur la Reproduction Artificielle du Rachitisme Chez Quelques Animaux, *Int. Zool. Congress*, Moskau, 1892.
11. LEHNERDT, F., *Beiträge für pathol. Anat.*, **46:** 468; **47:** 215, 1910; *Zeit. fur die gesante exper. Med.*, **1:** 175, 1913.
12. OEHME, C., *Beit. für path. Anat.*, **49:** 248, 1910.
13. PAPILLON, M. S., *Compt. rend. Acad. Sc.*, **71:** 320, 1870.
14. PECHER, C., *Proc. Soc. Exper. Biol. & Med.*, **46:** 86, 1941.
15. ROBINSON, R., LAW, K. A., AND ROSENHEIM, A. H., *J. Biochem.*, **33:** 66, 1936.
16. SCHOUR, I., HOFFMAN, M. M., SARNAT, B. G., AND ENGEL, M. B., *J. D. Res.*, **20:** 411, 1941.
17. SCHOUR, I., AND HOFFMAN, M. M., *J. D. Res.*, **18:** 161, 1939.
18. SCHOUR, I., HOFFMAN, M. M., AND SMITH, M. C., *Am. J. Path.*, **17:** 529, 1941.
19. SCHOUR, I., AND SMITH, M. C., *Univ. Ariz. Tech. Bull.*, No. 52, June 15, 1934 (Tuscon, Ariz., Univ. of Ariz.: 1934).
20. SCHOUR, I., AND PONCHER, H. G., *Am. J. Dis. Child.*, **54:** 757, 1937.
21. SHIPLEY, P. G., PARK, E. A., MCCOLLUM, E., SIMMONDS, N., AND KINNEY, E. M., *Johns Hopkins Hosp. Bull.*, **33:** 216, 1922.
22. SOBEL, A. E., GOLDFARB, A. R., AND KRAMER, B., *Proc. Soc. Exper. Biol. & Med.*, **31:** 869, 1934.
23. SOBEL, A. E., COHEN, J., AND KRAMER, B., *J. Biochem.*, **29:** 2640, 1935.

EFFECT OF SALIVA ON CAPILLARY PERMEABILITY[1]

B. M. LEVY, B.A., D.D.S., AND J. L. T. APPLETON, D.D.S., Sc.D.

School of Dentistry, University of Pennsylvania, Philadelphia, Pa.

A particulate dye, injected intravenously, normally escapes from the circulation relatively slowly and stains the tissues uniformly. Bouffard (1) and Goldman (2) were among the first to describe this phenomenon. In areas to which various irritants have been applied, the rate of diffusion increases to such a degree that these areas become deeply stained, establishing a clear contrast with the faintly colored background. The local staining of a test site in the skin of a rabbit has been employed as a means of demonstrating changes in capillary permeability (3).

EXPERIMENTAL

0.2 cc. of centrifuged human, mixed saliva was injected into the normal skin of a rabbit's abdomen. This was immediately followed by an intravenous injection of 7 cc. of 1 per cent trypan blue into the same animal. Within 5 minutes, a blue "blotch" appeared at the site of the saliva injection. Usually the blotch first appears as a blue ring around the border of the bleb, which rapidly takes on a solid blue color. The size of the bleb is about 1.5 cm. by 1.0 cm. A control intradermal injection of turpentine in the same animal caused the appearance of a similar blue blotch.

Saliva, *per se*, is not usually considered an irritant. We might consider the living bacteria normally found in saliva an irritant and, as such, the factor responsible for the increase in capillary permeability. To verify this assumption, the centrifuged saliva was filtered through a Jena sintered glass filter (G5/3). This bacteria-free saliva was injected into the skin of a rabbit, followed by an intravenous injection of trypan blue. The dye rapidly accumulated from the blood stream at the site of this saliva injection. We may therefore assume that there is a factor in saliva, other than a bacterial irritant, which is responsible for the increase in capillary permeability. In an effort to determine which factor or factors in saliva are responsible for this increase in capillary permeability, we eliminated substances known to be present in saliva, injecting the "residual" saliva into the skin of the rabbit. We tried to keep the unknown factors in saliva at a minimum by adding no chemicals to it. Sterile saliva contains salts, proteins, and enzymes. Filtered saliva was used in all of the following experiments.

We prepared a relatively salt-free saliva by dialyzing saliva through a cellophane membrane against distilled water for 24 hours at ice-box temperature, changing

[1] Read at the 19th General Meeting of the International Association for Dental Research, St. Louis, March 15 and 16, 1941 (J. D. Res. **20**: 281, 1941). Received for publication June 27, 1942.

the water at frequent intervals. The enzyme system was inhibited by heating saliva at 80°C. for 10 minutes. The proteins in a saliva sample were denatured by heating at 100°C. for 10 minutes. We extracted a sample of saliva with ether to remove ether-soluble substances.

0.2 cc. of each of the above mentioned saliva samples were injected intradermally into the previously depilatorized skin of a rabbit's abdomen. Control injections of untreated sterile filtered saliva, Ringer's solution isotonic with saliva, physiologic NaCl solution, horse serum diluted with Ringer's solution isotonic with saliva to the same protein content as saliva (0.5 per cent) and distilled water were made. These injections were immediately followed by an intravenous injection of trypan blue. The results of this work are shown in Table I. A positive result indicates a localization of trypan blue. This is taken as evidence for an increase in capillary permeability.

Parotid saliva was isolated and injected intradermally as the previous prepared samples were. The result of this experiment is also shown in Table I. The reaction was delayed, that is, the blue blotch appeared at the site of the injection

TABLE I

TEST SAMPLE	REACTION	TEST SAMPLE	REACTION
Dialyzed saliva	+	Diluted Ringer's	−
Saliva heated 100°C. 5 min	+	Diluted horse serum	−
Saliva heated 80°C. 5 min	+	Sterile distilled water	−
Saliva after ether extraction	+	Saliva steril untreated	+
Parotid saliva	+ (delayed)	Physiologic NaCl	−

of parotid saliva 5 minutes after a positive result was obtained with whole mixed saliva.

Whole stimulated centrifuged saliva was diluted with sterile distilled water to make solutions containing 80%, 50%, 20%, 10%, and 5% saliva. When injected intradermally a positive "trypan blue" test was found in all dilutions.

DISCUSSION

The above observations show that the factor or factors in saliva which are responsible for causing an increase in capillary permeability are not the salts, proteins, enzymes, ether-soluble substances, mucin, or the hypotonicity of saliva. The salts were ruled out by demonstrating that dialyzed saliva increases capillary permeability, while Ringer's solution, isotonic with saliva, and physiologic NaCl solution fail to increase capillary permeability.

The possibility of protein as the causative factor was eliminated by showing that the injection of saliva, after its proteins have been denatured, increases capillary permeability. On the other hand, the injection of a horse serum solution containing the same amount of proteins foreign to the rabbit as saliva, fails to produce this result.

The observation that saliva, heated to inhibit the action of its enzymes, in-

creases capillary permeability proves that enzymes are not the responsible factor. Ether-soluble substances were ruled out by the demonstration that saliva from which ether-soluble substances are removed still increases capillary permeability.

The fact that mucin-free (parotid) saliva gives a positive reaction, although slightly delayed, indicates that mucin, *per se*, is not responsible for the increase of capillary permeability.

Thus it is seen that a hypotonic solution, containing salts, proteins, and enzymes, will not cause an increase in capillary permeability. There must be another factor in saliva which produces this reaction. This factor must be highly active, since solutions containing only 5 per cent saliva, when injected intradermally, promptly cause an increase in capillary permeability.

This increase in capillary permeability is not necessarily saliva acting directly on the capillaries. The saliva may induce the tissues to release histamine or a histamine-like substance, which would be responsible for the increase in capillary permeability (3). From inflammatory exudates Menkin (4) isolated a substance (leukotaxine) which increases capillary permeability and attracts leukocytes. Saliva has both these properties as demonstrated by the present studies and by those of Dietz (5). The property of increasing capillary permeability is not removed from saliva by heating it to 100°C. or by extraction with ether. Leukotaxine also is thermostable at 100°C. and is insoluble in ether. In respect to diffusibility through cellophane, there may be a difference between leukotaxine and the saliva factor responsible for increasing capillary permeability: but the procedures employed for studying diffusibility were not strictly comparable.

By substituting saliva for the inflammatory exudate in Menkin's (6) method for the isolation of leukotaxine, we were able to obtain a crystalline material which, when suspended in distilled water and injected intradermally, induced prompt increase in capillary permeability.

This material was sent to Dr. Menkin to have its possible identity with leukotaxine verified. He reported to us (7) that the material was biologically identical with leukotaxine, but that microscopically the crystals did not look like the crystals of leukotaxine.

CONCLUSIONS

1. The intradermal injection of saliva results in a prompt increase in capillary permeability.

2. The factor in saliva which is responsible for this increase in capillary permeability is not a protein, salt, or enzyme.

3. There is a substance in saliva which, isolated in crystalline form, increases capillary permeability and is biologically similar to leukotaxine.

REFERENCES

1. Boufford, G., Injections des couleurs de benzidine aux animaux normaux; Etude experimentale et histologique. *Ann. de l'Inst Pasteur*, **20**: 539, 1906.

2. Goldmann, E., Die äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der vitalen Farbung. *Beitr. z. klin. Chir.*, **64**: 192, 1909.

3. Rocha e Silva, M., and Dragstedt, C. A., Observations on the trypan blue capillary permeability test in rabbits. *J. Pharmacol. and Exper. Therap.*, **73:** 405, 1941.
4. Menkin, V. Studies on inflammation. XII. Mechanism of increased capillary permeability. A critique of the histamine hypothesis. *J. Exper. Med.*, **64:** 485, 1936.
5. Dietz, Anne K., The chemotactic influence of human saliva upon leukocytes. *J. D. Res.*, **18:** 361, 1939.
6. Menkin, V., Studies on inflammation. XIV. Isolation of the factor concerned with increased capillary permeability in injury. *J. Exper. Med.*, **67:** 129, 1938.
7. *Idem*, Personal communication.

OPSONIN IN THE SALIVA OF CARIES-NEGATIVE AND CARIES-POSITIVE PATIENTS[1]

CAROLYN W. HAMMOND, M.A. AND JOSEPH P. WEINMANN, M.D.

From the Foundation for Dental Research of the Chicago College of Dental Surgery, Chicago, Ill.

The presence of opsonin in saliva has been described (1). It was noted in the 59 tests recorded in that paper that the percentage of phagocytosis showed an extremely great range for each of the 3 organisms used. There was a variation of from 0–30 per cent for *Sarcina lutea*, 0–38 per cent for *Streptococcus viridans* and 0–78 per cent for the oral lactobacillus. The patients from whom the saliva for these experiments was secured were selected at random and with no regard for the presence or absence of dental caries. However, a record was kept of the condition of the teeth. In tabulating the results it was noted that a correlation existed between the amount of caries and the percentage of phagocytosis. Saliva from persons with rampant caries did not stimulate phagocytosis to exceed 10 per cent while saliva from caries-free persons stimulated phagocytosis as much as 78 per cent.

As a result of this observation another series of experiments was carried out limiting the patients to those who were either caries-free or had rampant caries. The caries-negative patients were all dental students at the University of Illinois and were made available through the courtesy of Dr. M. K. Hine. Each person had received a thorough clinical examination, a radiograph had been made and he had been proved caries-inactive according to the tests of Hadley (2), Fosdick (3) and Blayney (4). The persons with rampant caries were secured through the director of the examination room of the Chicago College of Dental Surgery.

Blood was obtained from hospital patients by venous puncture and was oxalated. It was centrifuged slowly to induce the formation of a "buffy coat." The leucocytes were pipetted off and were washed 4 times in physiologic salt solution. Serum was secured from clotted blood drawn at the same time as the oxalated blood.

The organism used was an oral lactobacillus because it had shown the greatest range in phagocytosis. The lactobacilli were grown 24 hours in 1 per cent dextrose broth. They were centrifuged, washed twice in physiologic salt solution and were then suspended in 10 drops of salt solution.

Two to 3 cc. of unstimulated saliva were collected from each patient. The saliva was plated out on tomato agar (pH 5.0) according to the method of Hadley (2) as a check on its content of acid-producing bacteria. With one exception the caries-free persons were consistently negative and the rampant group positive.

[1] Presented at the 20th General Meeting of the International Association for Dental Research, New York, March 14–15, 1942. Received for publication June 30, 1942.

The technique for the tests was the same as that described in the first experiments (1).

Thirty-eight persons were used for the tests; 10 of these had rampant caries and 18 were caries-free. Of the caries-free persons 3 had several tests made, one individual was used 3 times and two were used 4 times each. The saliva of one individual who had consistently had negative tomato agar plate counts showed only 14 per cent phagocytosis the first time used. This was lower than any from the caries-free individuals had been previously. The tomato agar plate indicated that this saliva had become positive for acid-producing organisms. It remained positive for approximately 6 months and the 2 phagocytosis tests done during this time showed a drop in the index to 8 per cent. The fourth phagocytosis test, done 6 months later, showed a rise to 44 per cent. This rise coincided with the appearance of the first negative plate in 6 months.

Ten of the caries-free group had apparently been caries-free all their lives as they had no fillings and there was no evidence of caries by any of the known methods of examination. Eight of the caries-free group had been caries-positive at some time as each had at least one filling. However, they had been caries-inactive for a number of years as all the fillings were several years old and no evidence of caries activity could be found by any test. There was no difference in the phagocytic index of this group and the caries-free group without fillings.

Excluding the individual whose phagocytic index dropped to 8 per cent when he became positive for acid-producing bacteria, the indices ranged from 20–60 per cent with an average of 45.7 per cent for the caries-free group. The indices for the rampant group ranged from 0–10 per cent with an average of 4 per cent.

In the experiments previously reported (1) it was noticed that when both saliva and serum were used with organisms and leucocytes, the saliva apparently exerted a depressing effect on the opsonic activity of the serum. The more recent experiments show that the depressing effect is most marked in persons with rampant caries. Saliva from caries-free persons depresses the opsonic activity of serum very little. Hektoen and Ruediger (5) found that certain salts affect serum in such a way that an antiphagocytic effect is produced. Perhaps the presence of such a salt may explain the inhibitory effect on serum of saliva from persons with rampant caries.

In order to identify further the nature of the phagocytosis-stimulating factor dilution experiments were done using saliva from individuals who were caries-free. It was possible to dilute out this factor in saliva. A 1–10 dilution reduced it considerably; 1–100 reduced it further and 1–1000 to 1–1,000,000 inhibited it completely. This is in accord with the findings of Hektoen and Ruediger (5) who were able to dilute out the opsonin in serum.

SUMMARY

Thirty-five phagocytosis experiments were carried out using saliva from 18 caries-free individuals and 10 with rampant caries. The saliva from the caries-free persons stimulated 37 per cent of the leucocytes (average) to take up bacteria. Saliva from persons with rampant caries stimulated only 4 per cent of the leu-

cocytes (average) to take up bacteria. When both saliva and serum were used in the tests an inhibition of the opsonic action of the serum was noted. This inhibition was very slight when saliva from caries-free persons was used but was very marked when saliva from persons with rampant caries was used.

The authors wish to express their gratitude to Dr. L. Hektoen for his advice, suggestions and criticism.

REFERENCES

1. Hammond, C. W. and Weinmann, J. P., *J. D. Res.*, **21**: 279, 1942.
2. Hadley, F. P., J. D. Res., **13**: 415, 1933.
3. Fosdick, L. S., Hansen, J. L. and Epple, C., *J. A. D. A.*, **24**: 1275, 1937.
4. Blayney, J. R., Kesel, R. G. and Wach, E. C., *J. D. Res.*, **15**: 326, 1936.
5. Hektoen, L. and Ruediger, L. F., *J. Infect. Dis.*, **2**: 128, 1905.

THE EFFECT OF A LOW CALCIUM AND VITAMIN D FREE DIET ON THE SKELETON AND TEETH OF ADULT RATS[1]

AXEL P. LUND, PH.D., D.D.S., AND W. D. ARMSTRONG, PH.D., M.D.

Laboratory of Dental Research, University of Minnesota, Minneapolis, Minn.

Several investigations have shown that the histological structure and the chemical composition of the continually growing incisor tooth of the rat may be altered by deficient diets (1, 2, 4, 6, 7, 13). Toverud (14) investigated the influence of a calcium low diet and pregnancy on the calcification of bone and teeth of rats. The ash content of the femur diaphysis was greatly decreased, while that of the incisors and molars was 3.5 per cent and 2.0 per cent, respectively, below the average for the normal control rats. Schour (12) has concluded, from a review of his work and that of others, that the mineral phase of mature teeth of limited growth is not susceptible of alteration. However, it does not appear that the effect of a low calcium and vitamin D free diet on the composition of the bones and of the enamel and dentin of the separately examined incisor and molar teeth of adult rats has been studied.

EXPERIMENTAL

Twenty male albino rats were used in the present investigation. Their age of 11 months and their average initial and final weights of 332 and 343 gms. indicate that their molar teeth were fully developed at the beginning of the experimental period. All animals were given, for 5 weeks, the adequate or control diet (Table I) to adjust them to the experimental conditions. They were then divided into 2 equal groups. One group was continued on the control diet for 220 days and will be referred to as the control group, while the other group was given the experimental diet for an equal time. The 2 diets (Table I) were so constructed as to include equal amounts of all ingredients and elements except calcium and vitamin D. The calcium and phosphorus contents of the prepared diets, as determined by chemical analysis, is given in Table II. The animals were fed at a level of 10 grams of these prepared diets per day and the food consumption was determined daily. Distilled water was supplied *ad libitum*.

After the 5 week preliminary period the calcium and phosphorus ingested and the calcium and phosphorus in the combined excreta were determined for each ten-day period. To the excreta for each rat, collected in an 800 cc. Kjeldahl flask were added 100 cc. of concentrated hydrochloric acid, and the contents heated until a homogeneous mixture was obtained. This mixture was cooled to

[1] This work was supported by a grant from the Carnegie Corporation while one of us (A. P. L.) held a Carnegie Fellowship in Dentistry. Read at the 20th General Meeting of the International Association for Dental Research, New York, March 14 and 15, 1942 (J. D. Res., **21**: 335, 1942). Received for publication July 20, 1942.

room temperature and made up to a known volume. Aliquots of this solution were wet ashed according to the method of Neumann (9). Aliquots of the wet

TABLE I

Composition of the diets

	CONTROL DIET	EXPERIMENTAL DIET
	per cent	*per cent*
Egg white*	12.0	12.0
Salt mixture	4.4	2.773
Embo†	10.0	10.0
Cerelose	56.6	58.227
Corn oil	5.0	5.0
Crisco	12.0	12.0
Beta-carotine	1.6 gm./kgm. of food	1.6 gm./kgm. of food
Viosterol	0.2 gm./kgm. of food	1.6 gm./kgm. of food

Salt mixture for the control diet‡ (10)

$Ca\ Citrate \cdot 4H_2O$	154.83 gms.	A
$Ca\ (H_2PO_4)_2 \cdot H_2O$	56.62	
K_2HPO_4 (anhyd.)	109.86	
KCl	62.64	
NaCl	38.70	
$CaCO_3$	34.48	
$MaCO_3$	16.71	
$MgSO_4$	19.25	
$Ferric\ Citrate \cdot 1\frac{1}{2}\ H_2O$	94.18 gms.	B
NaF	3.68	
$MnSO_4$	1.17	
$K_2Al_2(SO_4)_4 \cdot 24H_2O$	0.67	
KI	0.30	

493.06 gms. of A mixed with 6.90 gms. of B constitutes the salt mixture used in the control diet.

Salt mixture for the experimental diet‡

KH_2PO_4 (anhyd.)	44.50 gms.	A_1
$NaH_2PO_4 \cdot H_2O$	16.25	
K_2HPO_4 (anhyd.)	109.86	
KCl	62.64	
NaCl	38.70	
$MgCO_3$	16.71	
$MgSO_4$ (anhyd.)	19.25	

307.91 gms. of A_1 mixed with 6.90 gms. of B, constitutes the salt mixture used in the experimental diet.

* Commercial egg white. Detoxified according to the method of Parsons (11).

† A General Mills Product rich in thiamine, riboflavin, and vitamin E.

‡ Reagent grade chemicals were used in preparing the salt mixtures.

ashed material were used for the determination of calcium according to standard methods and phosphorus according to the method of Fiske and Subbarow (3)

as adapted for the use of the Evelyn photoelectric colorimeter. The sum of the data obtained for each group per 10 day period was divided by the number of animals, thus giving average value for calcium and phosphorus excreted per rat per 10 day period. From these data, and from the data of food consumption, the mean intake and excretion of calcium and phosphorus for an average rat for an average 10 day period during the 220 days of observation employing the different diets was calculated and the standard deviations (Sx) computed. The data with regard to mineral balance are shown in Table III.

When the animals were sacrificed, the carcasses were boiled in water and the soft tissues removed by dissection. The humeri, femurs, molar and incisor teeth were cleaned and preserved in alcohol.

TABLE II

Calcium and phosphorus in the experimental and control diets

DIET	Ca	P	PER CENT Ca: PER CENT P
	per cent	*per cent*	
Experimental diet	0.007	0.451	0.015
Control diet	0.462	0.458	0.009

TABLE III

Average calcium and phosphorus ingested and excreted per rat for an average of 20 10-day periods together with the standard deviation of the means

Results in milligrams

GROUP	Ca	Sx	Ca	Sx	P	Sx	P	Sx
	Ingested		Excreted		Ingested		Excreted	
Experimental	6.8	± 0.58	33.7	±16.53	457.8	±38.68	423.5	±81.44
Control	455.2	±33.88	422.9	±42.45	405.0	±33.00	433.2	±65.58

During the removal of the teeth from the alveolar bone the following observations were made: In the experimental group the alveolar bone was very soft and friable. The alveolar crest was resorbed and the teeth were loose. The molar teeth of the members of the experimental group showed more wear of the occlusal surfaces than those of the control group.[2] In the control group, on the other hand, the alveolar crest displayed no resorption, and the teeth were firm in their sockets. The incisor teeth appeared to be as well developed in the experimental group as in the control group. The mean weight of the cleaned, fat extracted and dried incisor teeth of single animals was 0.4258 ± 0.0200 (Sx) and 0.4210 ± 0.0346 (Sx) gms. for the experimental and control groups respectively.

The long bones were rendered fat free by continuous extraction for 24 hours using a Soxhlet extractor with alcohol and ether as the solvent. They were

[2] This excessive attrition may have been due to occlusal trauma caused by the looseness of the teeth rather than to softness of the teeth.

dried in an oven at 110° for 12 hours, cooled and weighed. The left humerus was used to determine percentage of calcium and phosphorus, and calcium and phosphorus per cubic centimeter of bone. In order to determine the volume of the bone, a special pycnometer was designed, wherein mercury was used as a submersion medium. The determinations were made at a definite temperature in order that the weight of pycnometer filled with mercury would be a constant value, and that the volume of the mercury displaced by the bone could be calculated without applying corrections. Volume and denisty of the bones were calculated as follows:

$$\text{Vol. of bone} = \{[\text{Wt. of pycnometer full of Hg}] - [(\text{Wt. of pycnometer} + \text{Hg} + \text{bone}) - (\text{Wt. of bone}^3)]\} \text{ (times)}$$
$$\text{Vol. of 1 gm. of Hg at experimental temperature.}$$
$$\text{Density of bone} = \text{Vol. of bone/Wt. of bone before immersion in Hg.}$$

To test the accuracy of the method 5 determinations of the volumes of 3 humeri from different rats were carried out. The results in terms of cubic centimeters were as follows:

Bone	*Average volume ± Standard deviation*
1	0.122 ±0.0021
2	0.140 ±0.0005
3	0.139 ±0.0012

The average volume in cubic centimeters and the average density together with the standard deviation were determined for the left humeri for both the experimental and control groups. The results were as follows:

GROUP	AVE. VOL. ± Sx	AVE. DENSITY ± Sx
Experimental	0.198 ±0.0200	0.994 ±0.0646
Control	0.194 ±0.0220	1.111 ±0.0600

Each left humerus was dissolved in normal hydrochloric acid and made up to volume. Aliquots of this solution were used for the determination of calcium and phosphorus by the methods mentioned above. The percentages of calcium and phosphorus on the weight per cent basis and in terms of unit volume of bone were calculated. The average results, are given in Table IV.

The left femur from each animal, prepared for analysis as described above, was used for ash determination. The average results are as follows: Experimental group 65.21 ± 2.74 per cent, control group 65.83 ± 1.78 per cent.

The incisor and molar teeth were separately crushed, fat extracted and dried as described above and crushed in a diamond mortar until all particles passed through a 100-mesh sieve. The molar teeth from the experimental animals crushed easily, but vigorous pounding on the pestle with a hammer was neces-

[3] The weight of the bone after submersion in mercury was subtracted in the above equation rather than the true weight since a small but variable quantity of mercury may enter the porous bones.

sary to crush the molar teeth from the control animals. The friability of the incisor teeth of the 2 groups was not noticeably different. The enamel and dentin was separated into 5 fractions according to the method of Manly and

TABLE IV

Average contents of calcium and phosphorus in the humeri together with the standard deviations

GROUP	Ca	P	MGM. Ca/CC. BONE	MGM. P/CC. BONE
	per cent	*per cent*		
Experimental	25.3 ±0.88	11.74 ±1.07	251.0 ±17.35	115.6 ±13.18
Control	26.17 ±0.49	12.58 ±0.31	301.9 ±37.30	139.5 ±15.50

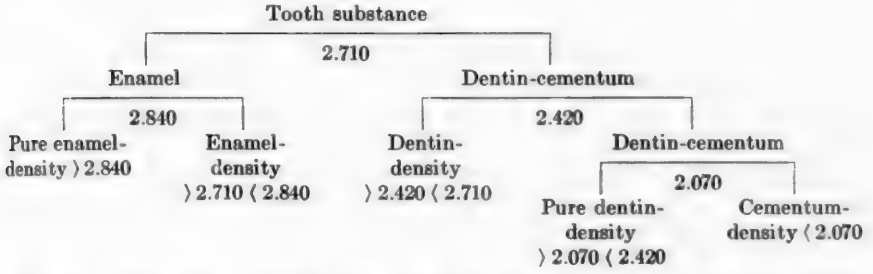


FIG. 1. Separation of powdered teeth into fractions according to density. Numbers enclosed within symbols ⟩ and ⟨ refer to the density limits of each fraction. Numbers not so enclosed refer to density of liquid employed at indicated place for separation of various fractions.

TABLE V

Average composition of fractions of incisor and molar teeth

DENSITY	EXPERIMENTAL ANIMALS		CONTROL ANIMALS	
	Ca	P	Ca	P
	per cent	*per cent*	*per cent*	*per cent*
Incisor teeth				
>2.093 <2.414	25.51 ±0.84	15.18 ±0.27	26.35 ±1.08	14.84 ±0.61
>2.414 <2.701	33.84 ±1.09	16.69 ±0.49	33.91 ±0.68	16.75 ±0.30
>2.701 <2.84	37.73 ±1.83	17.97 ±0.90	37.51 ±0.12	17.98 ±0.65
>2.840	38.41 ±0.16	18.29 ±0.88	38.46 ±0.75	18.24 ±0.15
Molar teeth				
>2.037 <2.425	30.23 ±0.93	13.79 ±0.33	30.24 ±0.60	13.79 ±0.79
>2.425 <2.711	35.40 ±1.44	16.53 ±0.45	35.42 ±1.42	16.82 ±0.63
>2.711 <2.841	36.18 ±0.80	17.07 ±0.39	36.53 ±1.09	16.90 ±0.40
>2.840	36.86 ±0.92	17.67 ±0.56	36.73 ±2.73	17.65 ±1.18

Hodge (8). A diagrammatic description of the course of the separations and the density limits of each fraction is shown in *fig. 1*.

These fraction of tooth substance were dried in the oven at 110° for 1 hour, and samples were weighed on a semi-micro balance, dissolved in normal hydro-

chloric acid and made up to volume. Aliquots of these solutions were used in determining calcium and phosphorus according to the methods listed above. The percentage of calcium and phosphorus was calculated together with the average values and standard deviation of the means for each group of animals. The results are shown in Table V. The indicated density limits of the fractions shown in Table V are the determined densities of the fluids used in the isolation of the fractions.

SUMMARY AND CONCLUSIONS

The influence of a diet very low in calcium and essentially vitamin D free, but otherwise adequate, on the calcium and phosphorus balance and on the composition of the skeleton and of the enamel and dentin of adult rats has been studied.

The animals on the experimental diet were in a negative calcium balance during the experimental period.

The molar teeth from the experimental rats were friable, but chemical analysis does not reveal any decalcification. The alveolar bone of these animals, however, was decalcified, the alveolar crest resorbed and the teeth loose in their sockets. The incisor teeth from the experimental rats grew and developed normally in weight and chemical composition.

The average ash content of the femurs of the experimental rats was not significantly lower than that of the control group.

The average density of the humeri from the experimental animals was decreased, but their volume was not altered. The percentage of calcium and phosphorus per unit weight of bone does not distinguish osteoporotic bones from normal bones, but calculations of weight of calcium or phosphorus or ash per unit volume of bone may serve to indicate the degree of osteoporosis.

The data indicate, under the conditions which produced osteoporosis of this experiment, that bone substance is lost from the interior of the bone without loss from the surface and that the mineral and organic phases are proportionally decreased in the osteoporotic bone.

REFERENCES TO LITERATURE

1. Becks, H., and Ryder, W. B., *Arch. Path.*, **12:** 358, 1931.
2. Downs, W. G. (Jr.), *J. D. Res.*, **12:** 363, 1932.
3. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, **66:** 375, 1925.
4. Gaunt, W. E., and Irving, J. T., *J. Physiol.*, **99:** 18, 1940.
5. Karshan, M., *Dental Science and Dental Art.* Edited by S. M. Gordon. (Philadelphia, Lea and Febiger: 1938) p. 105.
6. Karshan, M., *J. D. Res.*, **10:** 267, 1930; **11:** 64, 1931; **13:** 301, 1933.
7. Karshan, M., and Rosebury, T., *J. D. Res.*, **11:** 137, 1931; **12:** 437, 1932; **13:** 305, 1933.
8. Manly, R. S., and Hodge, H. C., *J. D. Res.*, **18:** 133, 1939.
9. Neumann, A., *Z. Physiol. Chem.*, **37:** 115, 1902-1903.
10. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, **34:** 131, 1918.
11. Parsons, H. T., *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.*, **97:** p. XXX, 1932.
12. Schour, I., *J. Am. Med. A.*, **110:** 870, 1938.
13. Templin, V. M., and Steenbock, H., *J. Biol. Chem.*, **100:** 209, 1933; **100:** 217, 1933.
14. Toverud, G., *J. Biol. Chem.*, **58:** 583, 1923.

HISTOCHEMICAL STUDY OF PHOSPHATASE AND GLYCOGEN IN FETAL HEADS[1]

N. H. HOROWITZ, PH.D.[2]

College of Dentistry, University of Southern California, Los Angeles and the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, Cal.

INTRODUCTION

Although there can be little doubt that bone phosphatase plays an important part in the formation of calcified structures in the body, its exact relationship to other determining factors is far from clear. The occurrence of the enzyme in tissues which never normally calcify (e.g., intestinal mucosa, kidney) clearly shows the inadequacy of any simple theory based on phosphatase alone. This is not surprising in view of the multiplicity of chemical factors—vitamins, hormones, and inorganic ions—known to influence the deposition of bone. Robison and coworkers, who have been outstanding in the field of phosphatase studies, have recognized for some time the existence of a "second mechanism" of calcification, the presence of which, in conjunction with phosphatase, is necessary for normal calcification. The "second mechanism" can be experimentally distinguished from the phosphatase mechanism by its sensitivity toward fluoride, iodoacetate, and glucose (1). The "second mechanism" has been the subject of numerous investigations, but its nature has not yet been elucidated.

One of the requirements of a theory of calcification is that it explain the source of the phosphoric esters which form the substrate of bone phosphatase. The small amount of organic acid-soluble phosphorus in the plasma (less than 0.5 mg. per cent) seems hardly sufficient to cover the requirement. Such a source of phosphoric esters would seem to be provided by the glycogen which has long been known to occur in cartilage (2) and which more recently has been shown also in the osteogenic tissue of endomembranous bone (3). This suggestion, first put forward by Harris (3), has recently received additional support. Glock (4) has demonstrated by chemical and histological methods that the glycogen of calcifying cartilage first increases to a maximum and then falls off as calcification proceeds. She has shown that in the long bones, glycogen is principally located in the region of the primary center of ossification, a point which has likewise been emphasized by Harris (5).

It is well known that glycolysis in muscle involves the degradation of glycogen through a series of phosphoric esters. Cori (6) has shown that the first step in the degradation consists in the cleavage of glycogen into glucose-1-phosphate, with the uptake of inorganic phosphate:

$$\text{glycogen} + H_3PO_4 = \text{glucose-1-phosphate}.$$

[1] Received for publication July 20, 1942.

[2] Present address, Biology Dept., Stanford University, California.

The reaction is catalysed by an enzyme, phosphorylase, and a co-enzyme, adenylic acid. It has recently been shown (7) that rabbit epiphyses contain a similar enzyme capable of carrying out phosphorolysis of glycogen in the presence of adenylic acid.

It is thus seen that a fair amount of evidence exists in favor of the glycogen hypothesis. Not the least attractive aspect of this view is that it may explain the nature of the "second mechanism" and its inhibition by substances known to poison the glycolytic process.

The purpose of the present investigation is to study the occurrence and localization of phosphatase and glycogen in the developing fetus, with special reference to the bones of the head. Such information, obtained at the same time on the same species—in some cases on the same animal—is of interest in connection with the postulated relationship of glycogen to phosphatase in calcifying tissues. Specifically, it is desired to know the distribution of phosphatase and glycogen in endochondral and endomembranous bones and in teeth; how the distribution changes during fetal development; and the relation, if any, between phosphatase and glycogen content and calcification.

METHODS

This study is based chiefly on fetal rats. The time of fertilization was determined by examining vaginal smears of the breeding females for spermatozoa. This was done daily, the fetal age being dated from the first occurrence of sperms in the smear. When the desired stage of gestation had been reached the animal was killed by a blow on the head, and the fetuses were immediately dissected out. The heads were removed and fixed in 95% alcohol. They were then run through dioxane and imbedded in paraffin. Serial transverse (coronal) sections were made at a thickness of 15μ. Particular attention was paid to the region containing the first molars, since these bear the closest resemblance to human teeth. In addition to the rat material, 3 fresh human fetuses (22, 31, and 33 mm.) were obtained and studied.

Phosphatase was determined by the method of Gomori (8). In this method, sections are incubated at 37°C. in a solution of na-betaglycerophosphate and calcium ion. Hydrolysis of the ester by tissue phosphatase results in the deposition, within the sections, of an insoluble calcium phosphate, which is subsequently converted to black cobaltous sulfide. The present author finds that the speed and sensitivity of the method are considerably increased by adding Mg ion to a final concentration of M/300 and buffering the solution at pH 9 with 0.5% veronal. These conditions are known to be optimum for bone phosphatase. The incubation period is 2 hours. The method does not distinguish between preformed bone and experimentally deposited calcium phosphate. For this reason, controls were run to determine the extent of calcification at each developmental stage. In most cases this was negligible in comparison with the amount of calcium phosphate found after incubation.

Glycogen was stained by the iodine method of Gage. This was checked in a number of instances by Best's stain and by the Bauer-Feulgen method. The results obtained by all three methods were in good agreement.

RESULTS

Rat fetuses were obtained at 13, 15, 17, and 20 days of gestation. Since phosphatase is destroyed by acid, a study of newborn animals was precluded by the necessity of decalcifying them before sectioning.

Some preliminary experiments were performed to show that phosphatase was actually what was being determined. It was found that sections which have been heated to 100°C. for a few minutes formed no calcium phosphate on incubation. Likewise, no reaction occurred when the phosphoric ester was omitted from the incubation medium. The pH optimum of the reaction was determined on sections of a 17 day fetus with the results indicated below. The density of the deposit in the region of the mandible is indicated by a number, 0 representing no reaction, 5 representing the maximum:

pH	*Density*
6 (acetate buffer)	0
7 (veronal buffer)	<1
8 (" ")	3
9 (" ")	5
10 (" ")	4

Incubated for 2 hours at 37° C.

It is clear from these data that bone phosphatase is involved in the reaction being considered.

13 day fetus. Glasstone (9) gives 13 days as the time of the beginning of tooth-bud formation in the rat. Fetuses obtained at this age were still in the gill arch stage, with no evidences of tooth-bud formation or other skeletal development. They were devoid of phosphatase. The author is therefore inclined to place the beginning of tooth-bud formation at the 14th day of fetal development, in agreement with Addison and Appleton (10).

15 day fetus. In the 15 day fetus, localized thickening and proliferation of the mouth epithelium mark the dental lamina. Calcification of the head has not yet started, but a strong phosphatase reaction is observed in several regions (*fig. 1*). These regions, in descending order of density of deposit are: prospective mandible, lateral aspect of prospective cranium, anterior part of prospective maxilla (i.e., premaxillary portion), lens epithelium (especially in the proliferative zone), rim of the optic cup, and the connective tissue layer of the villi of the choroid plexus. It is of interest to note that the prospective regions of calcification become chemically differentiated from the surrounding tissues before any apparent morphological differentiation of osteogenic tissue has occurred.

The test for glycogen is given by the following regions: prospective mandible, Meckel's cartilage, prospective premaxillary, lateral and ventral aspects of cranium, epidermis from the level of the eyes downward, epithelium of the ventral part of the nasal passages, and the epithelial cells of the choroid plexus.

17 day fetus. In the 17 day fetus the beginning of calcification is seen in the mandible, maxilla, and cranium. The dental lamina has invaginated to form the enamel organ, consisting of the inner and outer enamel epithelia, the enamel cord, and a central mass which is differentiating into the stellate reticulum. The

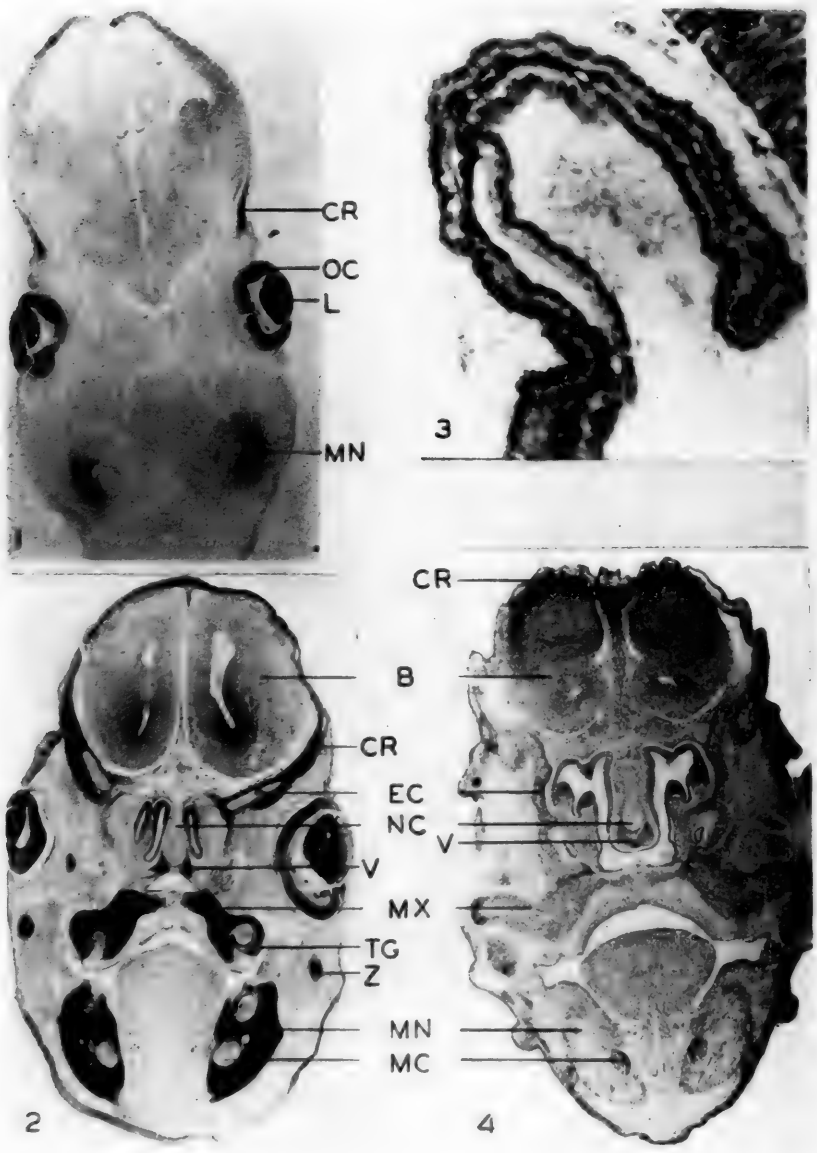


FIG. 1. Phosphatase in head of 15 day fetal rat. CR, cranium; OC, optic cup; L, lens; MN, mandible. Orig. mag. ×19

FIG. 2. Phosphatase in head of 17 day fetal rat. B, brain; CR, cranium; EC, ethmoid cartilage; NC, nasal cartilage; V, vomer; MX, maxilla; TG, tooth germ; Z, zygoma; MN, mandible; MC, Meckel's cartilage. Orig. mag. ×14

FIG. 3. Villus of choroid plexus of 17 day fetal rat, showing phosphatase in inner layer. Orig. mag. ×228

FIG. 4. Glycogen in head of 17 day fetal rat. CR, cranium; B, brain; EC, ethmoid cartilage; NC, nasal cartilage; V, vomer; MX, maxilla; MN, mandible; MC, Meckel's cartilage. Orig. mag. ×16.

dental papilla has formed in the concavity of the enamel organ; odontoblasts are not yet seen. The fibrous tissue surrounding the tooth-bud has arranged itself in parallel layers to form the dental sac.

The amount of phosphatase in the head has increased considerably (*fig. 2*). The phosphatase-containing areas around the mandible and maxilla have expanded from the centers present at 15 days to large areas of definite shape. In outline these areas resemble the bones which will later occupy them. Meckel's cartilage and the tooth germs of both upper and lower jaws are surrounded by masses of phosphatase-containing osteogenic tissue. In the upper jaw, these masses extend medially into the region of the prospective palate bones.

In the tooth-buds, the enzyme is found in the stellate reticulum, extending up into the dental lamina. In some cases it is also seen in the internal enamel epithelium; where this occurs, there is often a corresponding area on the underlying dental papilla. In the cranium, a layer of phosphatase-rich tissue is now found beneath the integument, covering the brain completely. Paired masses of phosphatase-rich cells are also found ventral to the nasal septum, where the vomer is beginning to calcify.

The enzyme is also found in several non-calcifying tissues. In the brain, it occurs in streaks both in the cortex and medulla. The fact that the choroid plexus is rich in phosphatase (*fig. 3*) suggests that the streaks represent blood vessels. In the epithelium of the dorsal surface of the tongue, small regularly spaced centers of phosphatase activity are found. These presumably bear some relation to the future papillae. The enzyme is also found in the hair follicles of the snout, where it is concentrated in the papilla and mesenchymal sheath.

While the phosphatase activity of the above tissues can be observed after a 2 hour incubation period, it was found that the enzyme can be demonstrated in practically all of the tissues if they are incubated long enough. This is in agreement with chemical investigations, which have shown that most mammalian tissues contain detectable amounts of phosphatase (11). It seemed of interest to determine the relative phosphatase activities of some of the tissues of the fetal rat head. This was done by measuring the time required for the deposition of a given density of calcium phosphate in the various tissues. The "standard" —or maximum density was that of the calcified trabeculae of the mandible. Consecutive sections at the level of the first molar were placed on individual slides and incubated with the substrate plus Mg ions at pH 9 for different periods of time. The calcium phosphate deposited in the tissues was then replaced by cobaltous sulfide, and the various regions were compared with the standard region. The time required for a number of tissues to attain "standard" density was as follows:

Tissue	*Hours*
Osteoblasts	½
Periosteal fibrous tissue of endomembranous bones and of ethmoid and sphenoid cartilages	1
Dental sac, lens epithelium	3
Stellate reticulum, dental papilla, brain capillaries	5
Tongue papillae	6
Cells of sphenoid, ethmoid, and Meckel's cartilages	30

In the 17 day fetus, the heaviest deposits of glycogen are to be found in the cartilaginous parts of the skull; namely, Meckel's cartilage, and the ethmoid and sphenoid cartilages (*fig. 4*). The nasal cartilage, however, is practically devoid of glycogen. The dental lamina and the adjoining mouth epithelium also give strong tests. In addition, glycogen is found in the calcifying regions (cranium, vomer, mandible, and maxilla) where it occurs in particles in the osteogenic fibers and along the free surfaces of the bone trabeculae. Among non-

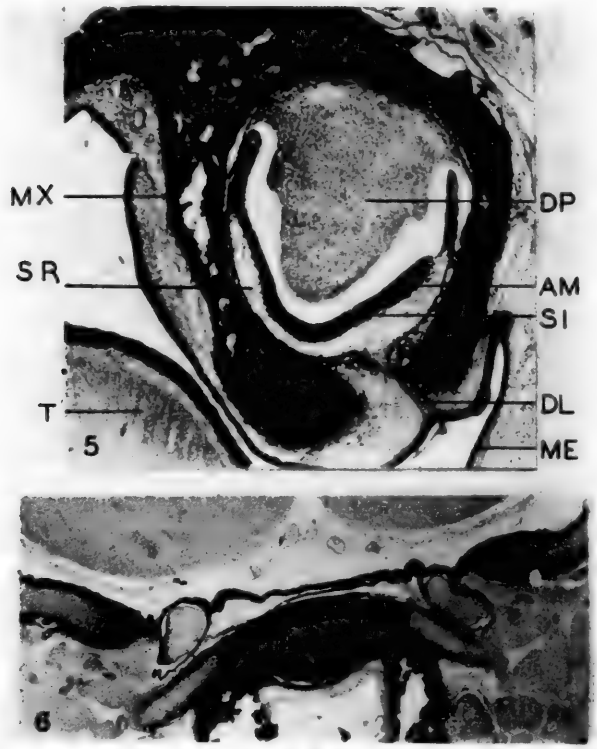


FIG. 5. Phosphatase in upper 1st molar of 20 day fetal rat. MX, maxilla; SR, stellate reticulum; T, tongue; DP, dental papilla; AM, ameloblasts; SI, stratum intermedium; DL, dental lamina; ME, mouth epithelium. Orig. mag. ×56

FIG. 6. Sphenoid cartilage of 20 day fetal rat, showing phosphatase within the cartilage at the onset of calcification. Orig. mag. ×30

skeletal tissues, glycogen is found in the muscle fibers of the tongue, in the epidermis, and in the choroid plexus.

20 day fetus. By the 20th day calcification of the head is well advanced, making sectioning often difficult. The tooth germs are considerably larger than at 17 days and cusp formation has begun in the molars. The enamel organ has differentiated into the ameloblast layer, stratum intermedium, stellate reticulum, and outer enamel epithelium. In the molars phosphatase is concentrated in the

stratum intermedium (*fig. 5*). In the incisors, where the formation of dentin and "osteo-dentin" has begun, phosphatase is found in the ameloblasts and odontoblasts. The dental pulp near the tip of the tooth is also rich in the enzyme.

The basal part of the sphenoid cartilage is now undergoing calcification. A layer of perichondral bone is found on the surface and spicules of endochondral bone are seen in the degenerating cartilage. Phosphatase is found not only in the perichondrium, but within the cartilage as well (*fig. 6*). The same is true of a small region of Meckel's cartilage just behind the symphysis. There has been a marked drop in the lens phosphatase, and an increase in tongue phosphatase, where the enzyme is found in association with the taste buds.

The glycogen distribution at this stage is qualitatively similar to that obtaining at 17 days. Quantitatively, there has been a drop in the glycogen content of those regions where calcification has proceeded most rapidly—i.e., mandible, maxilla, cranium, and basisphenoid. In Meckel's cartilage, the centrally located cells still stain deeply with iodine, while those on the periphery stain only slightly, if at all. In the molars, deposits of glycogen are found in the dental lamina and dental sac, but none in the ameloblasts or dental papilla. The incisors, however, show a heavily staining band of glycogen along the outer edge of the ameloblasts, particularly on the labial side of the tooth.

Human fetuses. For purposes of comparison with the rat material, the heads of 3 human fetuses of 22, 31, and 33 mm. length, respectively, were studied. Although the series is clearly incomplete, the findings may be briefly mentioned here, since a fuller study cannot be made at this time. The distribution of phosphatase and glycogen was found to parallel that observed in the rat head in all essential respects. Both substances are found in the osteogenic tissues of endomembranous bone (e.g., mandible), while in the cranial cartilages glycogen is contained in the cartilage and phosphatase in the perichondrium. The chief points of difference between the human and rat material lay in the phosphatase content of some of the soft tissues. The enzyme was not found in the choroid plexus of the human, nor in the lens. The tongue epithelium of the human fetuses contained glycogen, but not phosphatase, whereas the reverse is true of the rat. Large amounts of phosphatase, however, are found in the connective tissue of the human tongue. In the distribution of glycogen, the human heads were found to be almost identical with the fetal rat heads.

DISCUSSION

The histological findings are considerably simplified if the tissues of the head are divided into 3 classes with respect to their phosphatase and glycogen content, as follows:

1. Those which contain phosphatase, but not glycogen.
2. Those which contain glycogen, but not phosphatase.
3. Those which contain phosphatase and glycogen.

The discussion which follows will concern the rat, although the main conclusions apply equally to the human head.

In the first class fall the brain (except choroid plexus), the lens during the period of its rapid growth, the papillae of the tongue, and the hair follicles. In the second group are the muscle fibers and the epidermis. In the third class are all the calcifying tissues. The choroid plexus also contains both phosphatase and glycogen, but, unlike the calcifying tissues where the substances are distributed uniformly, they occur here in morphologically distinct layers. Glycogen is found only in the epithelium of the plexus, as has been known for many years (12). Phosphatase, on the other hand, is confined to the inner connective tissue layer. It is interesting to note that according to Stiehler and Flexner (13) a marked potential difference exists between these 2 layers. They ascribe this to the unequal distribution of indophenol oxidase between them. By maintaining the potential difference, the plexus is able to perform work in the secretion of the cerebrospinal fluid.

The only other non-calcifying tissue which contains both phosphatase and glycogen is the epithelium lining the angles of the mouth between the upper and lower molars. Here also the substances occur in different layers. Phosphatase is found only in the cornified superficial layer, whereas glycogen is confined to the deeper, living layer.

It is of interest to compare the situation in endomembranous bone with that in endochondral bone. In the former, which is the earliest to calcify, we find phosphatase and glycogen developing simultaneously, or nearly so, in the osteogenic tissue. The 2 substances appear to be uniformly distributed throughout the tissues, probably occurring in the same cells. As calcification proceeds, the glycogen and phosphatase content of the interior of the bone decreases, and the substances are concentrated mainly in the periosteum. In the parts of the skull which are preformed in cartilage, however, we find that up to the time when calcification begins the phosphatase and glycogen are separated, glycogen being contained within the cartilage, phosphatase in the surrounding connective tissue (perichondrium). At the onset of calcification, the barrier between the two tissues breaks down, and phosphatase is then found throughout the cartilage.

The situation in the teeth is not so clear as it is in bone. In the molars of the 20 day fetus, phosphatase occurs in the stratum intermedium and dental papilla, glycogen in the dental lamina and dental sac. Calcification of the molars does not begin until after birth, and hence was not covered in the present investigation. In the incisors, however, dentin and "osteo-dentin" formation begins on about the 20th day of gestation. By analogy with bone, it might be expected to find glycogen and phosphatase occurring at the site of calcification. This, however, was not observed. Phosphatase was found in the ameloblasts, odontoblasts, and dental papilla; glycogen in the dental lamina and at the base of the ameloblasts. Glycogen was not observed in the odontoblasts or dental papilla. It is possible that in the calcifying tooth glycogen is broken down too rapidly to be observed, or that phosphoric esters are transported to the sites of calcification from the glycogen stores in neighboring tissues. The fact that glycogen is found in association with the pre-functional ameloblasts is consistent with the former possibility.

In conclusion, it appears that in fetal heads phosphatase and glycogen occur together only in the cells of calcifying tissues. It seems highly improbable that this distribution is merely fortuitous. The findings thus support the hypothesis that both glycogen and phosphatase are involved in the calcification mechanism. As to the mechanism of the interaction, no conclusions can be drawn from the histological findings. All that can be said in this regard is that, whatever the mechanism, it involves the disappearance of glycogen from calcifying regions. At present, the most plausible scheme is that of the phosphorolysis-dephosphorylation mechanism described previously. It should be of interest to determine whether the present findings extend to adult tissues, as well, for the possible light they may throw on pathological calcification.

SUMMARY

1. Fetal rat heads from the 13th to 20th day of gestation were studied histochemically for phosphatase and glycogen.

2. The distribution of these substances at different fetal ages is described.

3. Three classes of tissue are distinguished: those which contain phosphatase but no glycogen, those which contain glycogen but no phosphatase, and those which contain both phosphatase and glycogen.

4. All the calcifying tissues belong to the third class, the incisors possibly excepted.

5. In non-calcifying structures which appear to belong to the third class it was found that the phosphatase and glycogen are in actuality separated into morphologically different layers.

6. Three human fetuses showed distributions of phosphatase and glycogen in essential agreement with the above findings.

7. It is concluded that both glycogen and phosphatase are involved in normal calcification, and the most probable mechanism of their interaction is discussed.

The author wishes to express his appreciation to Dr. Spencer R. Atkinson for his stimulating interest in this investigation, and to Mr. James Smith for taking the photomicrographs. Valuable assistance in the care of the animals was rendered by Mr. John Allan.

REFERENCES TO LITERATURE

1. Robison, R. and Rosenheim, A. H., *Biochem. J.*, **28**: 684, 1934.
2. Creighton, C., *Microscopic Researches on the Formative Property of Glycogen* (London, A. and C. Black: 1896).
3. Harris, H. A., *Nature*, **130**: 996, 1932.
4. Glock, G. E., *J. Physiol.*, **98**: 1, 1940.
5. Harris, H. A., *Bone Growth in Health and Disease* (London, Oxford Press: 1933).
6. Cori, C. F., *Cold Spring Harbor Symposia on Quantitative Biology*, **7**: 260, 1939.
7. Gutman, A. B. and Gutman, E. B., *Proc. Soc. Exp. Biol. and Med.*, **48**: 687, 1941.
8. Gomori, G., *Proc. Soc. Exp. Biol. and Med.*, **42**: 23, 1939.
9. Glasstone, S., *Proc. Roy. Soc. (London), B*, **126**: 315, 1938.
10. Addison, W. H. F. and Appleton, J. L., *J. Morph.*, **26**: 43, 1915.
11. Folley, S. J. and Kay, H. D., *Erg. d. Enzymforsch.*, **5**: 159, 1936.
12. Cowdry, E. V., *Special Cytology* (New York, Paul B. Hoeber: 1932), vol. 3, p. 1488.
13. Stiehler, R. D. and Flexner, C. B., *J. Biol. Chem.*, **138**: 603, 1938.

AMELOBLASTOMA: REVIEW OF 34 CASES[1]

JOSEPH L. BERNIER, D.D.S., M.S.[2]

Registry of Dental and Oral Pathology of the American Dental Association, Army Medical Museum, Washington, D. C.

There is little indication for detailed reference to the literature on ameloblastomas in view of the many excellent reports published (1, 2, 3, 4, 5, 6, 7). Microscopically, the ameloblastoma suggests an intimate association with epithelium which would ultimately possess the power of producing enamel. The varied histologic tendencies of this tumor are understandable when it is considered that it arises basicly from oral epithelium and thus may exhibit all of the embryonic features of that tissue. According to most investigators (5, 8) enamel is not produced in these tumors. Cases have been reported, however (10, 11, 12), in which amelogenesis is presumed to have occurred, but in those cases reviewed by the author one could not be impressed by such features. Two factors seem to be important in this respect. First, the degree of differentiation attained by the tumor cells at time of initial neoplasia; and second, the almost constant and presumably progressive degenerative changes within the stellate reticulum. These two factors, in varying proportions, seem to prevent other than an abortive attempt by the tumor to produce calcified ectodermal tissue.

The view held by many investigators (13, 14, 15) and apparently subscribed to by some others, that the ameloblastoma is closely associated with the basal cell carcinoma does not seem wholly tenable. The essentially peripheral characteristics of the basal cell carcinoma, together with its easily distinguishable morphological differences and clinical behavior, seem sufficient to place it in a separate category. In most instances there is little reason to confuse the histology of these two lesions as the degree of differentiation and specialization attained by the enamel epithelium, while not constant in each instance, is always sufficient to produce a tumor, the histologic features of which are usually unique.

Robinson (4) reported 17 cases from the literature in which "there seemed to have been present some evidence of malignancy." A review of the findings reported in many of these cases, together with other cases of suggested malignancy examined, would indicate that these features are not constant or striking enough to be of particular significance. It seems unfortunate, therefore, to attribute malignant characteristics to a lesion when such features have not been clearly demonstrated. Metaplasia toward the squamous type with the occasional production of pseudokeratin has been observed, but in the opinion of the author is not sufficient evidence of frank malignant change. Those cases with reported

[1] Received for publication June 29, 1942.

[2] Major, Dental Corps, U. S. Army, Secretary and pathologist to the Registry of Dental and Oral Pathology.

metastases do not bear close scrutiny. In most instances the histologic evidence on which this probability was based is not conclusive. It seems reasonable that the ameloblastoma, in most instances, grows by expansion rather than invasion, another feature suggesting its benign character.

There is little concrete information as to the etiology of this tumor. Its association with the odontogenic apparatus or with tissues which had the potentiality for producing the tooth germ is unquestioned. How early in the period of differentiation of the squamous cells of the oral epithelium that this tumor can occur is questionable. It is unlikely that the ameloblastoma could be produced before some indication of the morphological change in the cell had become evident. If this were true, the tumor would undoubtedly have a wider distribution. We can, therefore, limit the initial period of occurrence to an arbitrary period which begins after morphological changes in the cells of the oral epithelium have become evidence microscopically. Chronologically the tumor may form any time after this period has been reached. Embryonal ectodermal inclusions from the odontogenic apparatus in the form of rests undoubtedly account for the majority of ameloblastomas occurring after puberty. Obvious disturbances in the genesis of tooth formation, either through trauma or inflammation, are other likely factors. Other closely associated odontogenic lesions have been reported (17) as possible sources for this tumor.

A number of authors have reported series of cases, the majority of which have been included in Robinson's excellent review of the literature. There is a reasonably close correlation between most of the findings in these reports. However, there still exists the tendency by some to complicate what appears to be an otherwise simple tumor. To date the Registry of Dental and Oral Pathology of the American Dental Association has accumulated 34 cases of ameloblastoma, most of which have not been in any previous reports. It seems indicated, therefore, to review the findings in this group in an attempt to clarify some of the questionable statements. In most of these cases the history is rather complete and because of this it seems that the following information may be of some significance.

STATISTICS

It has been possible to check the diagnosis histologically of this series of cases, thus eliminating the possibility of including unconfirmed lesions. While a number of ameloblastomas of the pituitary gland and the tibia are included in the Registry, these cases have not been considered in the survey. In all of these cases the race, sex and age of the patients, together with a reasonably accurate location of the lesion, were included in the history. The average age of occurrence of these lesions was 38.3 years (*fig. 1*). This is in close agreement with Robinson's figure of 37.6 years. The age of discovery of the lesion, representing the age of occurrence less the duration, was 30.8 years, likewise in close agreement with Robinson and others. While this information is not of outstanding importance, it does indicate that the lesion is one of middle age. There is undoubtedly considerable inaccuracy due to the fact that the duration is based on

the questionable recollection of the patients. Of the 34 cases information as to duration was present in but 23. This figure averaged 5.57 years, somewhat

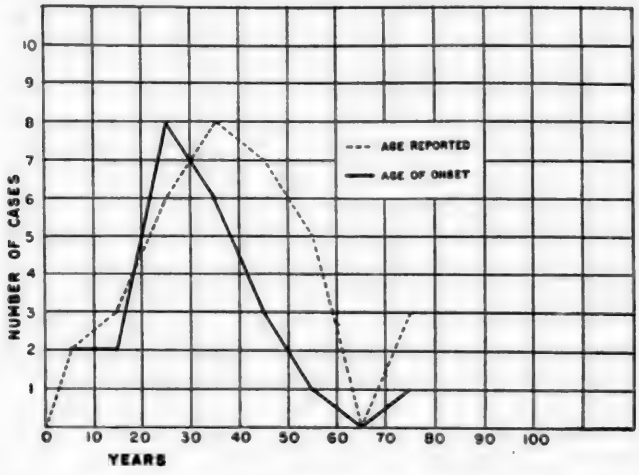


FIG. 1. Age reported and age of onset (average number of cases occuring in ten year periods)

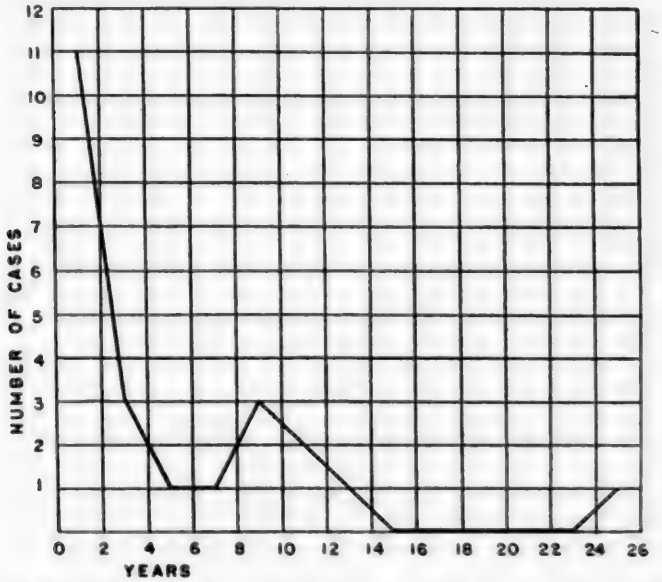


FIG. 2. Duration (average number of cases occuring in two year periods)

lower than previously reported figures (*fig. 2*). It is believed this figure is likewise of some significance in that it indicates the relatively slow growth of the lesion emphasizing its benign character. Twenty-five of the cases (75.3%) oc-

curred in white patients, and the remaining 9 cases (26.5%) in negro patients. This is in disagreement with the findings of Kegel who reported a much higher incidence in negroes (corrected ratio 11:1). Twenty-six of the cases (76.5%) occurred in males, and the remaining 8 (23.5%) occurred in females. This is in disagreement with Robinson's findings who reported 54.3% occurring in females. It is believed that these discrepancies can be accounted for in the small group of cases reported.

It has been rather definitely shown that the mandible is more frequently affected than the maxilla. This is borne out in this survey, 29 cases, (85.3%) occurring in the mandible, and 5 cases (14.7%) occurring in the maxilla. The distribution of the cases occurring in the mandible indicates that the angle of of the ramus is the most frequent site, 16 cases (55.1%) occurring on the right side in that location and 11 cases (37.8%) on the left side in the same region. Two cases (7.1%) occurred in the symphysis. Of the 5 cases in the maxilla some interesting observations could be made. These cases were evenly distributed, two being on the right side and three being on the left side. Of these 5 cases, 3 definitely involved the antrum and the remaining 2 had a questionable association.

Twelve (22.3%) of the cases gave definite history of trauma, the majority having as a background the extraction of one or more teeth. In one case there was an established endocrine disturbance, in another obvious trauma, and a third an infection involving an unerupted third molar. While many observers considered the ameloblastoma to be a painless tumor, 14 (41.1%) of those reported here gave a history of definite pain. In some instances this could be attributed to associated infections. In 12 of the cases (35.2%) resection of a portion of the bone was resorted to. In 20 cases, (58.8%) the tumor was removed surgically. In one instance the teeth in the area were extracted, the tumor adhering to one of the teeth. In the remaining one case the patient died from a pharyngeal obstruction. In this particular case the lesion was a recurrence which on two occasions, and at autopsy, had been diagnosed as ameloblastoma with epidermoid tendencies. A review of the material in this case indicates that perhaps the lesion in question may not have been ameloblastoma, but rather an undifferentiated squamous cell carcinoma. There were no metastases reported. Four of the cases in this group are reported as definite recurrences.

It may be seen that the majority of the findings in this survey are fairly consistent with those of other investigators. In reviewing the histology of these lesions it would seem that any attempt at classification could not be based on definite histologic principles. While both solid and cystic types of ameloblastoma have been recognized, I am of the opinion that the entire process is one of slow transition so that there is no dividing line separating these two varieties. It would appear, therefore, that little can be contributed to the information on these tumors by arbitrarily listing them in surveys of this kind as solid or cystic.

The ameloblastomas reported in this series varied from the simplest, purely ameloblastic unit to the undifferentiated pseudosquamous type, this latter form

exhibiting less constant behavior both clinically and histologically. It is important to state that it is not always possible to recognize this tumor by the clear-cut atypical columnar cells of the enamel epithelium, such things as hydropic degeneration, tumor necrosis, and the squamous metaplasia often masking the classical picture of enamel epithelium. It would appear that Kegel's (18) histologic grouping of this tumor into the adult form and the undifferentiated counterpart is in keeping with the usual findings.

The growth characteristics of the ameloblastoma indirectly determine the character of the tissues involved. Pressure of accumulating cystic fluid in the islets of epithelial cells is, on occasion, sufficient stimulus to produce a cystic lining of the transitional type of epithelium such as is seen in certain of the follicular cysts. It is believed by some that the production of this fluid is an abortive attempt at keratinization. There is little reason to believe that this process varies greatly from that seen in the epithelioma adenoides cysticum, in which case an amorphous colloid-like material is formed. Both of these lesions have their origin from the basal cell, the ameloblastoma arising only after differentiation toward the ameloblastic stage has begun.

The presence of secondary infection is apparently another factor influencing the behavior of the epithelial component of these lesions. In those exhibiting a severe purulent reaction one is apt to see a typical pseudo-epitheliomatous response sometimes tending to mask the underlying character of the lesion. I have seen such lesions confused with atypical basal and squamous cell carcinomas.

The details of the histology of this tumor are best considered by reviewing actual cases. The following cases are included in this report and are from the files of the Registry of Dental and Oral Pathology of the American Dental Association.

Case 1: A.M.M. Acc. 75597 (Contributed by Dr. M. G. Golden, Brooklyn, N. Y.).

History: A white male, age 52 years, who was first examined on November 8, 1938. An oral-antral sinus tract was noted on the left side. The patient states that this has existed for the last 25 years, at which time he had the upper left molar extracted by a barber. At intervals of 3 to 4 years particularly when he had a cold, a dark bloody discharge was noted coming from the oral opening of the sinus tract. This would persist for 2 or 3 weeks then apparently heal. At no time did he experience any pain nor was there any nasal discharge. X-ray examination revealed a markedly involved antrum on the left side. Transillumination showed the antrum to be completely dark. The nasal passages on both sides were clear. No evidence of tumor was noted in the nose or nasopharynx.

Operation: On November 12, 1938 a Caldwell-Luc operation was performed on the upper left antrum under local anesthesia. The entire antrum was completely filled with a tumor mass which was solidly impacted, homogeneous, smooth-surfaced and dark red in color. This was removed piecemeal as its size and consistency would not permit removal in toto through the operative opening.

Follow-up: The immediate post-operative course was good. He became and has remained symptom free. The window under the left turbinate has remained patent. Pre-operative sinus tracts persist, but there was no discharge.

Microscopic: The cellularity of this tumor is a prominent feature. The entire tumor

mass does not suggest invasive growth and appears to be contained within a loose, almost polypoid-like stroma, the outer surface of which is covered by a thin but essentially normal Schneiderian membrane (Plate I, *fig.* 1). Cilia are easily demonstrated. The tumor cells are arranged in irregular looping folds forming many clusters, many of which appear to be connected. In the center of these clusters a stringy, loose, and relatively acellular material suggesting stellate reticulum can be seen. In those areas where degeneration is not too advanced, the stellate shape of the cell can be seen. The cystic degeneration within these clusters is in most instances an early process. Degeneration is not a prominent feature at the present stage of neoplasia. There is an obvious uniformity in cell morphology, most of the peripheral cells being columnar in shape with a somewhat constantly positioned nucleus. These cells stain deeply and in the less cellular areas show evidence of abundant cytoplasm. Immediately adjacent to these peripheral cells there is, on the inner surface, a suggested "piling up" of a transitional type of cell suggesting the stratum intermedium. Scattered throughout the loose edematous background are inflammatory elements, many of which appear to be eosinophiles and larger mononuclear phagocytes. While vascularity is not a prominent feature, there are more than occasional blood vessels to be seen (Plate I, *fig.* 2). Some of these contain red blood cells. There are larger areas of extravasated blood showing partial organization. No calcified tissue is noted. The Masson stain indicates little or no collagen. No mucin can be demonstrated with the muci-carmine stain except in a few dilated glandular acini.

Case 2: A.M.M. Acc. 74998 (Contributed by Drs. Field and Ackerman, Newark, N. J.).

History: A 9-year old white female whose mother noted that the lower right first molar failed to erupt at the same time as the lower left first molar. However, in the absence of any symptoms nothing was done at this time. A few days before the patient was seen by the dentist the region of the lower right first and second molars became painful. Radiographs were taken revealing an unerupted first molar with its apex at the lower border of the mandible. The crown was surrounded by a dilated sac showing cystic degeneration. The second molar was absent entirely and in its place a radiolucent area measuring 3 x 2.5 cm. was noted. This was slightly irregular in outline and impinged upon the dilated sac of the first molar. No loculations were noted.

Operation: The mucous membrane was reflected and the bone over the buccal aspect of the first and second molars was removed. Over the crown of the first molar there was a cystic sac into which the crown of the tooth projected. There was no evidence of a tooth bud in the region of the second molar. In its place a tumor mass was noted. This was removed entirely together with a dilated follicular sac. The surrounding bone appeared essentially normal. Sections from the fibrous wall surrounding the crown of the first molar and from the tumor were submitted.

Microscopic: The follicular sac surrounding the first molar is a loosely arranged fibrous tissue showing evidence of degeneration. In some areas there appears to be considerable collagen. There are few fibroblastic nuclei noted and they are for the most part shrunken in size. Vascularity is not a feature. No inflammatory elements are noted. There is no epithelium in this material.

The tumor is composed of an embryonal mesenchymal matrix in which there are irregularly shaped collections of epithelial cells. Some of these collections are surrounded by a pink staining osteoid material which has included within its substance some of the epithelial cells. In one area there is calcification of this material (Plate I, *fig.* 3). Smaller foci of calcification are noted within the substance of some of these epithelial cells. Peripherally these cells are columnar in shape and are surrounded externally by a thin membrane and a wider clear zone in the connective tissue background. Some of the collections are very minute, consisting of hardly more than a dozen cells. There are occasional blood vessels to be noted. Cystic degeneration of the centrally placed cells is not a prominent feature, although in one area this has progressed moderately at the expense of the stellate reticulum (Plate I, *fig.* 4).

Case 3: A.M.M. Acc. 65091 (Contributed by Dr. P. Philip Gross, Philadelphia, Pa.).

History: A 60-year old white female who had an upper third molar extracted. There was a persistent post-operative opening into the antrum with an intermittent discharge of a purulent bloody material. No pain or other discomfort was noted at any time. At irregular intervals the patient complained of an itchy feeling in the region of the tuberosity. Both intra- and extra-oral radiographs disclosed a shadow apparently not communicating with the antrum. The clinical diagnosis was ameloblastoma.

Operation: No details as to the type of operation performed are available. Material was received for biopsy examination, following which the bulk of the tumor was removed and the diagnosis confirmed.

Microscopic: The section revealed a rather cellular tumor, the elements of which are well differentiated. There are irregular connecting strands of epithelial tissue, all of which are outlined by the columnar elements of the ameloblastic epithelium (Plate I, *fig.* 7). The stellate reticulum is particularly abundant, central cystic degeneration being noted only in occasional areas (Plate I, *fig.* 6). There are a few mitotic figures in evidence. Considerable extravasated hemorrhage surrounds much of the tumor mass. There is much hemosiderosis in this latter material. One does not see any evidence of squamous metaplasia, although there is a tendency for the undifferentiated portions of the stellate reticulum to arrange themselves in a whorling manner suggesting a very early attempt at keratinization. Some of the tumor cells bear a marked resemblance to the basal cells of the epidermis. There is a scattered inflammatory reaction in the intervening connective tissue septa which consists of small lymphocytes, plasma cells and occasional granular leucocytes.

Case 4: A.M.M. Acc. 62204 (Contributed by Dr. P. Philip Gross, Philadelphia, Pa.).

History: A 73-year old white male in good general health. He noticed a lump under the tongue immediately behind the anterior teeth. At time of examination the patient stated this had been present for approximately two months. There was no discomfort at any time. Clinically a hard area was noted directly beneath the anterior portion of the tongue and immediately posterior to the symphysis of the mandible. X-ray examination was made.

Operation: Under a local anesthetic the teeth in the involved area were removed, considerable tumor tissue adhering to some of the extracted teeth. The remaining tumor was removed by curettement. The diagnosis was ameloblastoma.

Microscopic: The outstanding feature of this lesion is its similarity in certain areas to squamous epithelium. Many of the cells exhibit frank squamous characteristics such as well developed intracellular bridges and the formation of keratin. The peripheral cells, however, tend to retain their columnar characteristics, although some alteration in their morphology is evident. There are many areas of necrosis in the center of the nests of tumor cells. The fibrous tissue stroma is young and the cells appear to be quite active. There is a diffuse inflammatory reaction consisting in some areas of solid collections of lymphocytes and plasma cells and in other areas numerous leucocytes, many of which are eosinophilic. There is much hemorrhage. This lesion has many frank epidermoid characteristics and demonstrates the malignant potentialities of the ameloblastoma (Plate II, *figs.* 1 and 2).

Case 5: A.M.M. Acc. 28623 (Contributed by Army Medical Museum, Washington, D. C.).

History: A 51-year old colored male who when first seen had a tumor the size of an orange on the left side of the face in the region of the ramus of the mandible. The patient stated that for the past 10 years this mass had been slowly enlarging, but that at no time was there any pain or other symptoms. X-ray examination revealed a multiloculated cystic area involving the ramus and a portion of the body of the mandible. The buccal plate of the mandible had been almost completely destroyed. There was no history of trauma.

Operation: Under a local anesthetic the tumor was removed piecemeal. Resection of the mandible was not resorted to.

Microscopic: The advanced cystic degeneration of this lesion is a feature (Plate II, *figs.* 3 and 4), although there are areas of solidly packed islets of ameloblastic epithelium, some of which exhibiting practically no central cystic degeneration. This is a well differentiated tumor. One has no difficulty in recognizing the ameloblastic cells, the stratum intermedium, or the stellate reticulum. Mitotic figures are absent. Some of the cystic areas contain an eosinophilic staining material suggesting keratin. There is some hemosiderin pigment contained within large phagocytic cells. The intervening fibrous stroma is moderately collagenous and contains a perivascularly distributed chronic inflammatory reaction. This appears to be an actively growing lesion, although well within benign limits.

Case 6: A.M.M. Acc. 51505 (Contributed by Walter Reed Gen. Hosp., Washington, D. C.).

History: A 24-year old white male who was admitted to the hospital with a diagnosis of central follicular cyst, vertical and horizontal ramus, mandible, right, due to unerupted right third molar. Clinical examination revealed a crepitant cystic area in the right side of the mandible involving most of the ramus and the posterior portion of the body. This tumor had been constantly painful. The patient exhibited a typical Frohlich's syndrome. The Wassermann and Kahn reactions were negative. Radiographs revealed an extensive cystic destruction involving the ramus and a portion of the body of the mandible. The sella appeared normal.

Operation: The tumor was removed without resorting to resection of the mandible. During the following month deep X-ray therapy was directed to the site of the tumor. The amount of X-ray is not stated.

Microscopic: There is considerable fibrous tissue surrounding the islets of ameloblastic epithelium. The fibrous tissue, in close association with the tumor elements, exhibits a basophilic degeneration. The tissue is rather adult and vascularity is not a prominent feature. The ameloblastic islets show central degeneration of the stellate reticulum with a slight tendency toward squamous metaplasia. Some of the islets contain a granular debris and a fairly well formed keratinous material. Mitotic figures may be identified, although they are not plentiful. There is apparently some individual cell dyskeratosis. There is no outstanding inflammatory reaction noted (Plate II, *fig.* 5).

SUMMARY

In this review of 34 cases of ameloblastoma one is impressed with the extreme variability of the histology of this tumor. To arbitrarily list it as either solid or cystic does not seem warranted insofar as the greater percentage of lesions seems to be in the stage of transition between these two extremes. The findings as to the age of discovery, the age at time of report, and duration are in agreement with the statistics reported by other authors.

It is important to recognize that the ameloblastoma, while it may have malignant potentialities as evidenced histologically, is essentially benign in its clinical behavior. An appreciable percentage of these tumors will show epidermoid tendencies up to a certain point. However, metastasis is an uncommon occurrence. In those cases in which this has been reported, reviewed by me, the ameloblastic features were lacking.

There seems little conclusive evidence for associating this tumor with the well differentiated basal cell carcinoma. While these lesions may have a common point of origin, the germinal layer, and certain other similar growth characteristics, they are still essentially different tumors. One cannot depend entirely upon the cell morphology, ignoring the clinical features.

Recurrence in this lesion is an important consideration. The belief of McFarland that mixed tumors of salivary gland origin are more easily removed in their entirety when the lesion has reached an appreciable size seems applicable to the ameloblastoma. In those cases in which too much bony structure has not been

SUMMARIZED DATA

AGE REPORTED	AGE OF ONSET	RACE	SEX	DURATION	SITE		SYMPTOMS	POSSIBLE ETIOLOGY	TREATMENT	REMARKS
					Max.	Man.				
3	—	W	M	—		L	None	—	Removal	
9	7	W	F	2 yrs		R	Pain	—	Removal	
17	15	W	M	1½ yrs.		R	Pain	—	Removal	
18	10	W	F	8 yrs.	R		Pain	Extr.	Removal	
19	17	W	M	2 yrs.		R	Sw.	Trauma	Removal	
22	21	W	M	1 yr.		R	—	—	Removal	Recurrence
23	22	W	M	1 yr.		R	—	Extr.	Removal	
24	—	W	M	—		R	Pain	Endo.	Removal	
25	25	C	M	3 mo.		L	Disch.	Extr.	Removal	
25	21	C	F	4 yrs.		R	Sw.	—	Resection	
27	25	W	F	1½ yrs.		R	None	—	Resection	
31	21	W	M	10 yrs.		R	Pain	—	Removal	
32	—	C	M	—		Rs†	Pain	—	Removal	
33	33	C	M	2 mo.	La*		Pain	Extr.	Removal	Recurrence
36	—	W	M	—		Ls	—	—	Resection	
36	26	W	M	10 yrs.		L	—	—	Resection	
38	—	W	M	—		R	—	—	Removal	
39	35	C	M	4 yrs.		L	—	Inf.	Resection	
39	36	W	M	3 yrs.		R	—	—	Removal	
41	—	C	M	—		R	—	—	Removal	
42	41	W	M	1 yr.		L	—	—	Removal	
44	31	W	M	13 yrs.		R	Pain	—	Resection	
45	—	W	M	—	R		Pain	—	Resection	Recurrence
45	33	W	M	12 yrs.		L	Pain	Extr.	Removal	
45	45	C	F	6 mo.		L	—	—	Removal	
49	39	W	F	10 yrs.		L	Pain	—	Died	Recurrence
51	—	C	M	—		L	None	—	Resection	
52	27	W	M	25 yrs.	La*		Pain	Extr.	Removal	
54	48	W	M	6 yrs.		R	Sw.	—	Resection	
56	—	W	M	—		R	—	Extr.	Resection	
60	—	W	F	—	L		Pain	Extr.	Removal	
71	—	W	F	—		R	—	—	Resection	
71	59	C	M	12 yrs.		Ls	Pain	—	Resection	
73	73	W	M	2 mo.		Rs	Sw.	Extr.	Removal	

* Antrum.
† Symphysis.

destroyed the chances for complete removal are greater if the lesion is sufficiently large to permit satisfactory operative procedure.

The ameloblastoma, contrary to the belief of many, is not necessarily a painless lesion. This is undoubtedly because its most usual site of occurrence is

at the angle of the ramus and the body of the mandible where it may easily become secondarily infected. The presence of a suppurative inflammatory process may influence somewhat the reactivity of the component of the tumor resulting in a pseudo-epitheliomatous hyperplasia.

The incidence of an inflammatory reaction within the tumor mass was sufficiently high in this group to mark it as an almost constant occurrence. In some instances the exudate was scanty, and predominantly chronic in character. The age of the fibrous tissue component of these tumors varies proportionately with the age of the tumor. In those of long standing considerable collagen is produced which tends to diminish the vascularity in these areas.

REFERENCES

1. Kegel, R. F. C., *Arch. Surg.*, **25**: 498, 1932.
2. McFarland, J., and Patterson, H. M., *D. Cosmos*, **73**: 656, 1931.
3. Simmons, C. C., *Annals Surg.*, **88**: 693, 1928.
4. Robinson, H. B. G., *Arch. Path.*, **23**: 831, 1937.
5. Robinson, H. B. G., *Arch. Path.*, **23**: 664, 1937.
6. Bump, W. S., *Surg., Gynec. and Obst.*, **44**: 173, 1927.
7. Murphy, J. T., *Radiology*, **3**: 377, 1924.
8. Thoma, K., *Oral Pathology* (St. Louis, C. V. Mosby: 1940).
9. Figi, F. A., and Stafne, E. C., *J. A. D. A.*, **21**: 1822, 1934.
10. Bernays, A. C., *M. Record*, **28**: 1, 1885.
11. Chibret, A., *Arch. de med. exper. et d'anat. path.*, **6**: 278, 1894.
12. D'Aunoy, R., and Zoller, A., *M. J. Record*, **130**: 274, 1929.
13. Sprawson, E., and Keizer, W. R., *D. Record*, **53**: 369, 1933.
14. Krompecher, E., *Beitr. z path. Anat. u. z. allg. Path.*, **64**: 165, 1918.
15. Tratman, E. K., *Brit. D. J.*, **59**: 417, 1935.
16. Bernier, J. L., *J. A. D. A.*, **28**: 1779, 1941.
17. Cahn, L. R., *D. Cosmos*, **75**: 889, 1933.
18. Bernier, J. L., *Annals Dent.*, N.S. **1**: 21, 1942.
19. Geschickter, C. F., *Amer. J. Cancer*, **24**: 277, 1935.

PLATE I

Fig. 1. Case 1. Respiratory epithelium is shown at *A*. Note loose polypoid stroma *B*, and irregular islands of tumor tissue at *C*. Similarity to stellate reticulum is clearly evident in this area.

Fig. 2. Higher power of case 1. Blood vessels in loose mesenchymal stroma are shown at *A*. Irregular columnar character of the ameloblastic epithelium is clearly evident at *B*.

Fig. 3. Case 2. Loose mesenchymal (pseudo-osteoid) tissue at *A*, has undergone calcification at *B*. Ameloblastic tumor tissue is seen at *C*, in which foci of calcification are present.

Fig. 4. Higher power of Case 2. Islets of tumor tissue *A*, are contained in loose mesenchymal stroma. Degenerating areas of stellate reticulum *B*, have progressed to frank cystic areas at *C*.

Fig. 5. Tumor elements *C*, and the stroma *D*, (Case 2) are shown in more detail in this higher power photomicrograph.

Fig. 6. Case 3. Abundant stellate reticulum *A*, is shown in contrast to ameloblastic cells *B*. Chronic inflammatory exudate is seen at *C* and connective tissue stroma at *E*.

Fig. 7. Higher power of Case 3. Ameloblastic character of tumor cells *A*, may be clearly seen.

PLATE I

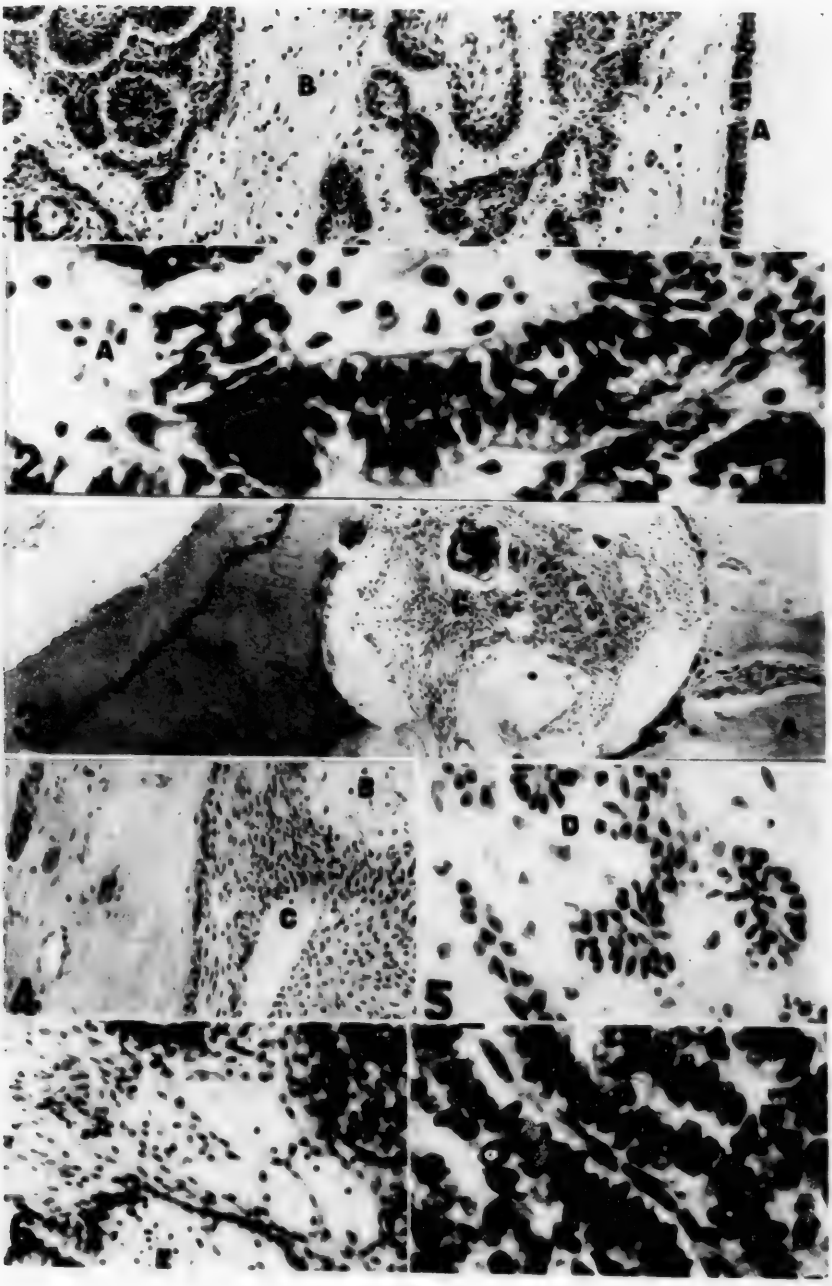


PLATE II

FIG. 1. Case 4. Squamous character of tumor is clearly shown at *A*, and *B*.

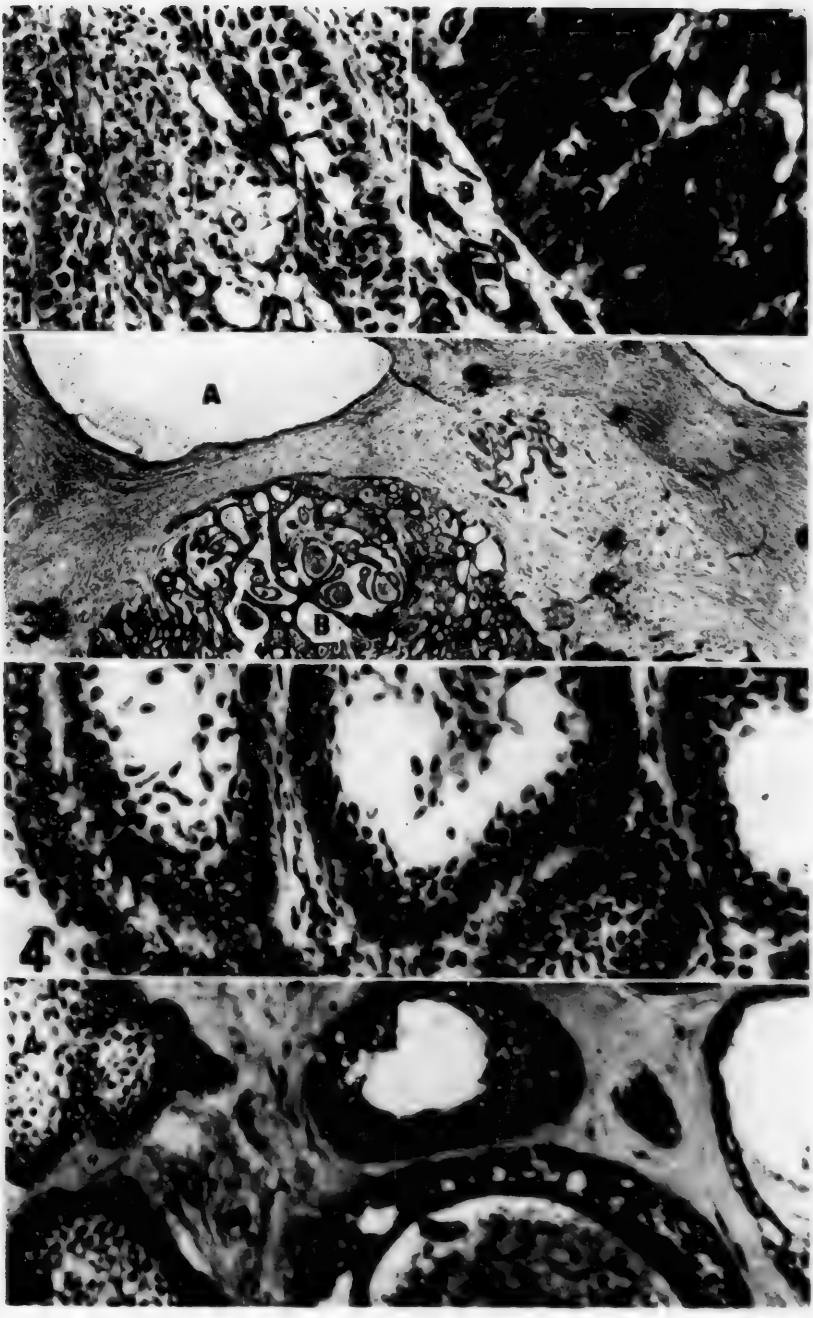
FIG. 2. Higher power of Case 4. Note pleomorphism of cells shown in higher power at *A*. Character of stroma may be seen at *B*.

FIG. 3. Case 5. Combination of cystic areas *A*, and solid tumor at *B*, are shown in this section.

FIG. 4. Case 5. Section from another area of same tumor showing well differentiated features of solid portions. Stellate reticulum *A*, ameloblastic cells *B*, and connective tissue stroma *C*.

FIG. 5. Case 6. Cystic degeneration of stellate reticulum is prominent in this case. More nearly normal stellate cells are shown at *A*, and large cystic space containing keratinized granular debris at *B*. Connective tissue stroma is seen at *C*.

PLATE II



CULTIVATION AND ISOLATION OF MOUTH SPIROCHETES[1]

OTTO W. WICHELHAUSEN, M.D. AND RUTH H. WICHELHAUSEN, M.D.

From the Department of Pathology and Bacteriology, Johns Hopkins University, Baltimore, Md.

INTRODUCTION

Complete bacteriological studies of material from cases of periodontosis, ulcero-membranous gingivitis and tonsillitis demonstrate a mixed flora of spirochetes, fusiform bacilli and various combinations of other organisms representing the normal mouth flora. With the exception of the spirochetes and fusiform bacilli none of the various organisms is found dominant or constantly present in the different clinical forms of disease. The constant presence and predominance of the spirochetes and fusiform bacilli gives them a certain etiological significance, and histological examination of the diseased tissues suggests strongly that there is a fusospirochetal infection, since these organisms, especially the spirochetes, are found deep in the tissues, often in advance of actual necrosis (1, 4, 24).

Successful experimental infections of animals have frequently been accomplished with material from mouth lesions (3, 5, 6, 8, 9). Such methods give only suggestive but no definite evidence of the pathogenicity of the individual organisms involved. The need for experimenting with pure cultures or with mixtures of pure cultures in order to obtain conclusive results has been pointed out by Smith (5), Proske and Sayers (9) and Seguin and Vinzent (10). Smith reports that pure cultures of the individual members of the flora found in Vincent's infection are non-pathogenic to the laboratory animals used, but that characteristic abscesses may be produced with culture mixtures containing *Spirocheta microdentium*, fusiform bacilli, a vibrio and an anaerobic streptococcus. Inoculation of a mixture of any 2 or 3 of these organisms did not result in a lesion. The findings of Smith are supported by Proske and Sayers who worked with cultures obtained from pulmonary fusospirochetal lesions. Seguin and Vinzent found 3 strains of *Spirocheta microdentium* slightly pathogenic for guinea pigs and obtained characteristic abscess formation when these cultures were injected together with a culture of one other organism (*B. fusiformis* or *"Bacillus of Sordelli"*).

Most of the organisms found in periodontosis or ulcero-membranous lesions may be cultivated, isolated and identified with comparative ease. The obstacle to working with pure cultures has been the difficulty to obtain pure cultures of the mouth spirochetes and to identify them properly once they have been isolated.

[1] Received for publication July 29, 1942.

Successful cultivation and isolation of mouth spirochetes was reported by Mühlens and Mühlens and Hartmann in 1906 (11, 12), and later by other workers such as Noguchi (13, 16), Seguin and Vinzent (10), Smith (4, 5), Rosebury (14) and others (15, 17, 18, 19, 20, 25). All authors agree that growth of the mouth spirochetes can be obtained only under anaerobic conditions in media containing some enriching substance such as ascitic fluid, serum or blood, whereas the addition of fresh tissue does not seem to be an absolute requirement for all strains. The isolation of the spirochetes has been attempted by many different methods such as centrifugation (21), prolonged incubation (10), selective media (10, 18), and most frequently by growth in solid media (10, 11, 12, 13, 14, 15, 16, 17). Mühlens (11, 12) and Shmamine (15) preferred to cultivate the spirochetes in deep agar shake tubes. Noguchi (13, 16) used the method of stab inoculation of agar tubes, and most of the later workers have employed some modification of Noguchi's method. Bacterial growth frequently remains confined to the stab whereas the spirochetes may migrate into the periphery from where they may be isolated through repeated transfers. Robinson (20) has reported isolation of mouth spirochetes by growing them through filter paper, and Kolmer and Kast (19), using Robinson's method for purification of their strains have isolated mouth spirochetes in liquid media by using high dilutions and selective media. Recently Rosebury and Foley (14) have obtained spirochete growth in small Petri plates, containing a very thick layer of a solid medium.

Little is known about the biological properties of the mouth spirochetes and even the strains that have been isolated in pure culture have been identified largely on the basis of their morphological characteristics. The fact that such identification is inadequate has been pointed out by Akatsu (22) who tested several spirochete strains for their saccharolytic activity and by Vinzent and Seguin who determined the saccharolytic and proteolytic activity and the serological relationship of the strains that they had succeeded in isolating (10).

Material for this study was obtained from patients of the dental clinic of the Johns Hopkins Hospital, in whom the clinical diagnosis of periodontosis, ulceromembranous gingivitis or Vincent's infection had been made. Care was taken to obtain the material from the depth of the lesions. In lesions such as are found in cases of gingivitis, the material was obtained with sterile cotton swabs. In cases of periodontosis a small platinum spud, which could be introduced easily between the teeth and the gingiva, was used. If possible a fairly large amount of material—enough to give a rather heavy turbidity when suspended in 1 cc. of beef heart infusion broth or physiological salt solution—was used. This material was, preferably within 1 hour after it had been obtained, injected into guinea pigs and more recently inoculated directly into culture media.

PRODUCTION OF ABSCESSES IN GUINEA PIGS

It has been shown by Smith, Rosebury and others (2, 3, 5, 6, 25) that subcutaneous inoculation of patients' material into the groin of a guinea pig will result in an abscess which contains spirochetes in large numbers along with a variety of bacteria. Kline (2) has pointed out that such abscesses cannot be

interpreted as a reproduction of the typical necrotic fusospirochetal lesions in man, and that the characteristic necrosis is obtained only if mechanical trauma of the tissues is applied in addition to the inoculation of spirochete material. Whatever the interpretation of the lesions may be, the production of guinea pig abscesses may be used to advantage as a preliminary step in the cultivation of mouth spirochetes in artificial media.

Material from guinea pig abscesses usually contains fewer of the gas-forming organisms that are frequently found in mouth lesions and which make the cultivation in artificial media difficult. After an abscess has developed the pus may be used for the inoculation of culture media or small amounts of pus (0.1 to 0.5 cc.) may be injected into other guinea pigs. Usually several guinea pig passages are necessary to eliminate the gas-forming organisms, and sometimes it is impossible to eliminate them by this method.

In addition to the possibility of eliminating the gas-forming organisms, the production of abscesses in guinea pigs is a useful means to preserve material containing spirochetes for future cultural work. Provided fresh material and a sufficiently large amount, 0.25 to 0.5 cc. of the broth suspension, are used for the guinea pig inoculation, there is usually little difficulty in producing such abscesses. However, failures occur, especially in the hot summer months, a fact that finds an interesting parallel in the difficulty in infecting rabbits with syphilis during the summer.

It is generally recommended that guinea pigs be injected subcutaneously or intramuscularly in the groin, taking care to avoid the peritoneum (3, 25). We have used this method but have lately preferred to inject the animals in the external muscles of the thigh. Abscesses of the groin occasionally perforate into the peritoneum or cause an ascending cellulitis of the abdominal and thoracic wall, whereas the abscesses of the thigh tend to remain localized.

It should be emphasized however that a microscopic preparation of guinea pig pus and of patients' material may present different pictures. In a typical case of Vincent's infection several morphological forms of spirochetes are found corresponding to *Treponema microdentium*, *T. macrodentium*, *T. buccale* and *T. vincenti* as described by Noguchi (23), *T. vincenti* being the predominant organism. In guinea pig pus we have found the majority of the spiral organisms to be small, regularly coiled spirochetes (*T. microdentium*) whereas the other morphological forms were present in negligible numbers or not at all. Similar findings were reported by Rosebury (3) but Proske and Sayers (25) have found the same morphological forms in guinea pig pus as in patients' material. It appears that *T. microdentium* is the mouth spirochete that is most easily maintained in animal passages, just as it is the only mouth spirochete, the successful cultivation of which has been repeatedly reported. Cultivation of *T. macrodentium* (4, 16, 19) and *T. buccale* (4, 25) has been reported less frequently and *T. vincenti* apparently has not been obtained in pure culture.

CULTIVATION AND ISOLATION IN ARTIFICIAL MEDIA

Our efforts to cultivate mouth spirochetes in artificial media and to isolate them in pure culture have been made with material from guinea pig abscesses

and more recently with material from mouth lesions. If such material is inoculated into a liquid medium spirochete growth may be obtained, but the spirochetes are far outnumbered and frequently completely overgrown by the accompanying bacteria. We have therefore tried to purify our cultures, patients' material and guinea pig pus, by filtration and by growth in solid media.

Filtration Method: It has been shown by Noguchi (7, 23) that the spirochetes, *T. pallidum* and *T. pertenue*, though not filtrable in the customary sense of the word, will grow through Berkefeld filters. Filter cylinders Chamberland-Pasteur, porosity L_3 and L_5, were used for our filtration experiments. Ordinary test tubes of a diameter of 18 mm. were heated, approximately at the junction of the lower and middle third and both ends of the tubes were pulled apart gently, in order to obtain a constriction of the tubes of about 7 mm. in diameter and 1 cm. in length. The tubes were plugged with cotton, sterilized and filled with a beef heart infusion broth (Medium I, Table II) to a level 2 mm. above the constriction. The filters, previously sterilized in separate test tubes, were soaked by filling them with beef heart infusion broth. After 15 minutes any remaining broth was removed with a capillary pipette and the filters were then introduced with sterile forceps into the broth tubes. They were held in position

TABLE I

FILTERS USED	RESULTS: CONTENTS OF FILTRATE				TOTAL NUMBER OF FILTRATION EXPERIMENTS
	No organisms	Motile bacilli	Spirochetes and bacilli	Spirochete[5] only	
Chamberland-Pasteur L_3	13	1	6	18	38
Chamberland-Pasteur L_5	9	0	1	3	13

by the constriction, the lower 2 to 4 mm. of the filter being immersed in the medium. The filters were inoculated with 0.5 to 1 cc. of material containing spirochetes by means of a capillary pipette. Thick material, i.e., pus from a guinea pig abscess, was diluted previous to this operation with a small quantity of the beef heart infusion medium. The tubes were incubated at 37°C. in Brown or Brewer anaerobe jars, employing hydrogen. The filtrate was examined under the darkfield as soon as there was the slightest indication of growth, i.e. a faint turbidity, otherwise approximately every 48 to 72 hours. If no organisms were found the filters were reincubated over a period of 2 weeks. The results obtained are summarized in Table I.

It was found that the spirochetes may pass the filters as early as 24 hours and as late as 10 days following inoculation, the average time required for passage being between 40 and 72 hours. In many instances the spirochetes did not pass the filter at all. If the passage time exceeded 4 days the filtrate containing spirochetes was in a number of cases found contaminated with bacteria, usually tiny motile bacilli. Twenty-one pure cultures of mouth spirochetes were obtained by this filtration method, but these cultures did not remain viable for more than 2 or 3 days, and we have been unable to recover subcultures for more than 1 or 2 generations.

Cultivation in Deep Agar Shake Tubes: Since the cultivation of mouth spirochetes in solid media proved to us a more reliable method, the use of filters for the purification of spirochete material was temporarily abandoned. We have however resumed the filtration experiments in combination with solid media and will report on those later in this paper.

A liquid medium containing beef heart infusion broth, ascitic fluid and cysteine hydrochloride is a widely used medium for the cultivation of spirochetes. We found that mouth spirochetes in mixed culture would grow well in such a medium (Medium I, Table II) which also supported the growth of several laboratory strains of *Treponema pallidum*, the English and the German Reiter strains and the Nichols and Noguchi strains.

In an attempt to find a suitable solid medium for mouth spirochetes we carried out some preliminary experiments with pure cultures of *Treponema pallidum* (Reiter, Nichols, Noguchi) rather than with mixed cultures of mouth spirochetes. It was found that the addition of small amounts of agar to Medium I (Medium II, Table II) resulted in improved spirochete growth and that on addition of 0.6, 0.8 and 1 per cent agar to Medium I (Medium VII) growth of these spirochetes in isolated and typical colonies could be obtained.

We used the method of stab inoculation of the agar tubes only in the beginning of our work because we found it preferable to use deep agar shake tubes. The melted agar was inoculated at a temperature of 42°C., the inoculum was well mixed and the tubes were incubated in anaerobe jars at a temperature of 37°C. As a rule visible growth occurred 5 to 15 days following inoculation. The inoculation of the melted agar results in isolated colonies whereas by stab inoculation growth is obtained only along the stab. Another advantage is that it is possible to measure the amount of inoculum more accurately.

The same medium (Medium VII) was inoculated with mixed cultures of mouth spirochetes and with material from guinea pig abscesses or mouth lesions, and the mouth spirochetes grew in the same typical colonies as did the *T. pallidum* strains.

The spirochete colonies have an average diameter of 0.5 to 1.5 mm. They appear cloudy due to their very poorly defined outlines. When such a colony is fished as a whole and examined under the darkfield, the center represents a solid mass in which no details can be distinguished, whereas in the periphery entangled masses of spirochetes are seen from which individual organisms radiate into the surrounding agar. These spirochetes are frequently non-motile but successful transfers have proven their viability.

In very young cultures spirochete growth appears just as a slight haze which is difficult to see and which may be overlooked unless the tubes are examined under oblique light. The fully developed spirochete colonies are macroscopically easily visible and are easily recognizable in a mixed culture because their diffusiveness distinguishes them from the more distinct and well outlined colonies of the ordinary bacteria.

The colonies may be fished from these agar shake tubes with a capillary pipette. When a fresh medium is inoculated, the agar column in the pipette may

be expelled and broken off just above the spirochete colony, and usually a pure culture may be obtained after a limited number of subcultures. The subcultures

TABLE II

Growth of spirochetes on various media

NUMBER OF MEDIUM	BEEF HEART INFUSION BROTH		ASCITIC FLUID 10%	RABBIT BLOOD	DEXTROSE 0.5%	CYSTEINE HYDROCHLORIDE 0.1%	SODIUM THIOGLYCOLLATE 0.1%	AGAR %	AGAR 1% +1.5% DOUBLE LAYER PL	RESULTS
	pH 7.4	pH 6.5								
I	x		x			x				+
Ia		x	x			x				+
II	x		x			x		.05		++
IIa		x	x			x		.05		++
III	x			5%						++
IIIa		x		5%						++
IV	x			5%		x				+++
IVa		x		5%		x				+++
V	x			5%		x		.05		++++
Va		x		5%		x		.05		+++
VI	x		x					1.0 sh		++
VIa		x	x					1.0 sh		+
VII	x		x			x		1.0 sh		++++
VIIa		x	x			x		1.0 sh		++
VIII	x		x				x	1.0 sh		+++
IX	x		x					1.25 sh		++
IXa		x	x					1.25 sh		+
X	x		x			x		1.25 sh		+++
Xa		x	x			x		1.25 sh		+
XI	x		x					1.5 sh		++
XIa		x	x					1.5 sh		+
XIb	x		x					1.5 pl		+
XII	x		x			x		1.5 sh		++
XIIa		x	x			x		1.5 sh		+
XIIb	x		x			x		1.5 pl		+
XIII	x		x			x			x	++
XIV	x			10%				1.5 pl		++
XV	x			10%		x		1.5 pl		++
XVI	x			10%	x			1.5 pl		++
XVII	x			10%	x	x		1.5 pl		+
XVIII	x			10%					x	+++

sh = deep agar shake tube. pl = plate.

Liquid media: +, 1 to 3 spirochetes per field; impossible to maintain pure subcultures; ++, 2 to 5 spirochetes per field; impossible to maintain pure subcultures of most strains; +++, 5 to 10 spirochetes per field; possible to maintain pure subcultures; ++++, 10 to 40 spirochetes per field; good stock culture medium.

Solid media: +, poor growth obtainable; results very irregular; ++, satisfactory growth obtainable; results irregular; +++, good growth in 60 to 80 per cent of all cultures; ++++, abundant growth in 90 to 100 per cent of all cultures.

may be made in either solid or liquid media. Our usual procedure is to transfer a whole spirochete colony to a liquid medium and to use varying amounts of

this broth (one loop to several drops) as inocula for the agar medium. In addition, one whole colony is transferred to an agar shake tube, taking care to break up the colony as much as possible. This latter procedure is followed because a very large inoculum is frequently necessary to obtain spirochete growth. This is especially true for spirochetes from original material and for the first generations in artificial media. The need of a large inoculum is one of the factors that make the isolation of mouth spirochetes particularly difficult. A large inoculum of spirochete material from the mouth necessarily involves the inoculation of a large number of the rapidly and profusely growing bacteria. Since it is unpredictable how large the inoculum from patients' material must be to result in spirochete growth, it is advisable to use at least 6 to 10 inocula of varying size (1 loop to 1 drop of the broth suspension) for the initial cultures in artificial media. Usually 1 to 3 of the cultures will show spirochete growth, and the one which seems most promising is chosen for subsequent transfers.

The isolation of strains of mouth spirochetes from heavily or even only moderately contaminated shake tubes is a procedure that requires a certain amount of technical skill and usually involves a number of subcultures until a pure culture is obtained. We have attempted to overcome these difficulties by 2 methods: 1. the combination of the above described filtration method with cultivation in a solid medium, and 2. the cultivation of the mouth spirochetes in plates rather than in shake tubes.

Combination of Filtration and Growth in Solid Media: For the filter method the same constricted tubes as described above are used. The tubes are filled with Medium VII which is kept liquid until the filter, previously soaked with beef heart infusion broth, is introduced into the tube. The lower 2 mm. of the filter should be immersed in the agar. A young, impure spirochete culture or a suspension of spirochete material is pipetted into the filter and the tubes are then incubated in an anaerobe jar.

If the spirochetes have grown through the filter their presence in the agar is indicated by the appearance of a slight haze in the medium immediately surrounding the filter. From such cultures pure subcultures in liquid and solid media may be easily obtained. With this medium we have not encountered spirochete cultures of low viability as described above. If, as is occasionally the case, the spirochetes are accompanied by motile bacilli, the bacterial growth in the agar is slight and subsequent isolation of the spirochete strains is much facilitated.

Cultivation in Blood Agar Plates: Gates (26), Fortner (27, 28) and Aksjanzew-Malkin (29) have reported the cultivation of *T. pallidum* and of a mouth spirochete on the surface of blood agar plates. In spite of many attempts we have only once obtained growth of *T. pallidum* (Strain Kasan) on the surface of a blood agar plate, and only twice have we seen surface growth of the mouth spirochetes on blood agar (Strain S 26). The colonies of the Kasan strain measured 1 mm. in diameter and appeared greenish grey by transmitted light. There was no hemolysis. Viewed from the surface they appeared very delicate, well outlined, causing a slight depression in the agar rather than being elevated

above its surface. Only the center of the colonies showed a distinct knob-like elevation (*fig. 1*). The growth of the mouth spirochetes appeared as an extremely delicate, colorless film on the surface of the blood agar plates. Isolated colonies could not be distinguished and hemolysis was not apparent. However

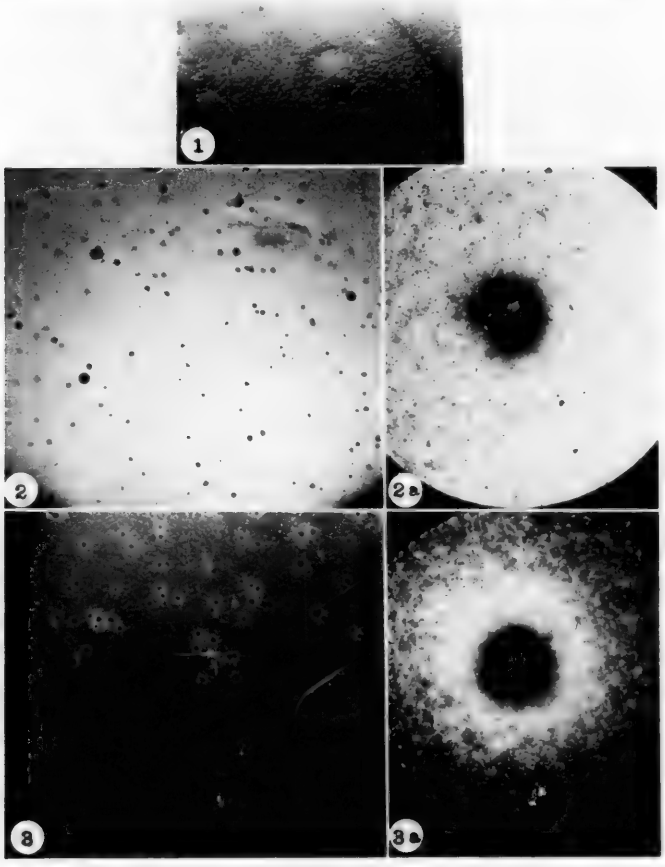


FIG. 1. Surface colony of *Treponema pallidum* (Kasan) on blood agar plate.
FIG. 2. Mixed culture of bacteria and mouth spirochetes in blood agar (entire plate hemolyzed). Natural size.
FIG. 2A. Spirochete colony of *fig. 2*. ×37.
FIG. 3. Pure culture of mouth spirochetes in blood agar plate. Natural size.
FIG. 3A. Spirochete colony of *fig. 3*. ×17.

it has been possible to cultivate mouth spirochetes and several strains of *T. pallidum* in the depth of blood agar plates.

To 10 cc. of 1.5 per cent beef heart infusion agar, pH 7.3 to 7.5, was added 1 cc. of defibrinated rabbit blood (Medium XIV, Table II). The medium was inoculated with spirochete material at a temperature of 45 to 46°C., and the

plates were incubated in anaerobe jars for at least 5 days and sometimes as long as 3 weeks.

Spirochete colonies in such blood agar plates are characteristic, so that they may be recognized easily and distinguished from bacterial colonies in a mixed culture (*fig. 2*). They measure usually 0.5 to 1 mm. in diameter, but may reach a diameter of 1.5 mm. They are always very poorly defined and never show the distinct outlines of bacterial colonies (*fig. 2a*). When they first develop they are sometimes so delicate that they might be overlooked, but they may be detected by examining the plate under oblique light. If the plate is reexamined 3 or 4 days later the colonies are easily visible and are surrounded by a zone of equally poorly defined partial hemolysis, the average diameter of the hemolytic zone being 1.5 to 2 mm. (*figs. 3, 3a*). A few of the blood cells in this zone appear intact, but fixed corpuscles are never encountered, so that the hemolysis may be described only as a partial one, not corresponding to either the typical alpha or beta types of hemolysis, produced by streptococci.

Eighteen strains of mouth spirochetes and 3 strains of *T. pallidum* (Strains Noguchi, Nichols and Kasan) have been cultivated in the depth of blood agar plates. All of them have shown the same characteristic cloudy colonies surrounded by a zone of partial hemolysis. It has therefore not been possible to use their appearance in blood agar plates as a means of differentiation between the strains. If the plates had been exposed to prolonged incubation, or if spirochete growth in them was heavy, the hemolysis was not always apparent, the plates being either completely hemolyzed or showing no hemolysis at all. Replating of such apparently non-hemolytic spirochetes has demonstrated all strains thus far examined to be hemolytic under suitable conditions.

It should be stated however that growth of the mouth spirochetes in these blood agar plates was not obtained with the same regularity as it was in the shake tubes containing Medium VII. If parallel cultures were made in the deep agar shake tubes and in blood agar plates, the results obtained in the shake tubes were three to four times as good as in the plates, i.e. negative plate cultures corresponded to positive growth in shake tubes in a number of instances and the spirochete colonies in shake tubes were always more numerous than in the plates.

In an attempt to explain the discrepancy of these results several factors should be considered: 1. Medium VII contains cysteine hydrochloride. Addition of cysteine hydrochloride to the blood agar (Medium XV) did not improve the results. 2. Medium VII contains ascitic fluid whereas blood was used for the plates. Addition of ascitic fluid (Medium XIb) instead of blood to the 1.5 per cent beef heart infusion agar resulted in similar or even poorer growth than that encountered in blood agar plates. Parallel cultures in liquid media showed that in general mouth spirochetes prefer media containing blood to those containing ascitic fluid. 3. The shake tubes were ordinarily inoculated at a temperature of 42°C., whereas a medium with a 1.5 per cent agar concentration requires inoculation at 45 to 46°C. Shake tubes containing Medium VII were inoculated with corresponding inocula at 42 and 45°C. and identical results were obtained in either culture. 4. The establishment of an immediate and complete anaero-

biasis in the shake tubes may be of influence. The difference in anaerobic conditions could be partly overcome by the placing of the cultures in the anaerobe jar as early as possible following inoculation. It was observed occasionally that shake tubes showed no growth in the upper 2 to 4 mm. of the medium. This layer corresponds to the depth of agar in plates, and failures to obtain satisfactory spirochete growth in single layer plates may be attributed at least in part to the lack of an immediate anaerobiasis. 5. The 1 per cent agar medium used in the shake tubes is too soft for plating purposes. We originally used 1.5 per cent agar for the plate cultures in order to obtain plates that could be inverted during incubation. Control cultures were made in shake tubes containing 1 per cent, 1.25 per cent and 1.5 per cent agar (Medium VII, X, XII). It was found that mouth spirochetes may grow in the 1.25 per cent and 1.5 per cent agar, but that growth is obtained with less regularity in the higher agar concentrations. We have therefore more recently inoculated 1 per cent beef heart infusion agar with spirochete material, poured the plate and after the medium had solidified, covered it with a layer of 1.5 per cent beef heart infusion agar in order to hold the softer agar in place. Blood was not added to the surface layer so that transparency of the medium would be maintained. This method which combines the more favorable 1 per cent agar medium with improved anaerobic conditions has given us much better spirochete growth than the 1.5 per cent single layer plates, although the results are not yet identical to those obtained in the shake tubes. In these double layer plates the colonies present the same appearance as in the single layer plates described above. Corresponding results were obtained when Medium VII was poured into plates and covered with a layer of 1.5 per cent beef heart infusion agar (Medium XIII).

Nineteen pure cultures of mouth spirochetes have thus far been isolated from solid media, either from Medium VII in shake tubes or from blood agar plates. Nine of them were obtained from guinea pig abscesses, ten from material taken directly from the patient. All these strains have certain common characteristics but may be distinguished from each other by differences in growth requirements and morphology.

MORPHOLOGY

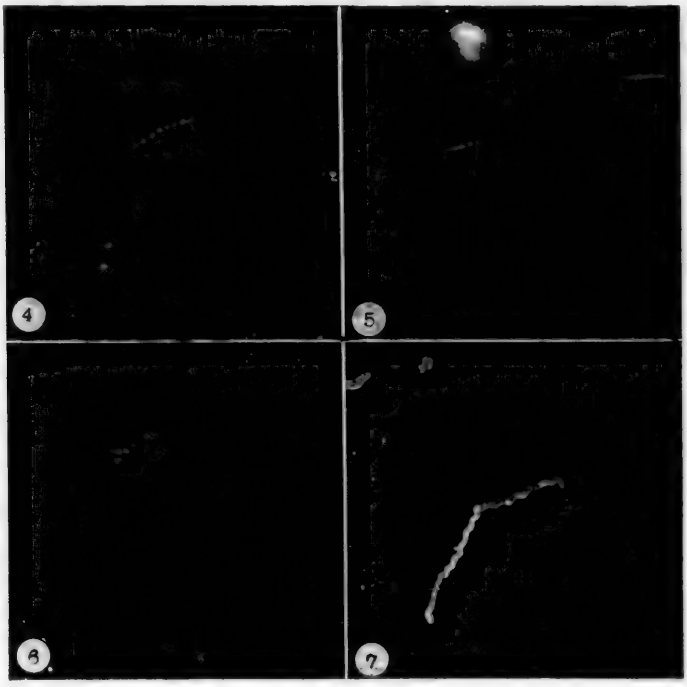
Since we have found initial spirochete growth as early as 3 days and as late as 21 days following inoculation, it is impossible to consider a spirochete culture of a certain age as a basis for morphological description. However certain morphological features have been observed with such regularity that they may be reported as typical for these strains.

All strains with the exception of one (S 84) are tightly and regularly coiled spirochetes, the younger cultures containing organisms with 6 to 10 spirals, whereas in the older ones very long forms with as many as 40 spirals may be observed. None of our strains seems to be the delicate, loosely and irregularly coiled organism described by Vincent and so frequently found in original material.

Fifteen of the strains usually appear as delicate spirochetes with 6 to 10 regular

tight coils. Their average length is 8 to 15 microns, their diameter 0.3 to 0.6 micron. Representative organisms of this group of spirochetes are shown in *figs. 4, and 5.* In a motile culture the spirals appear a little looser and less regular than shown in the illustration. The spirochetes show a certain though not a very pronounced flexibility. Movements are chiefly rotating, but, especially in solid media, a slow serpentine motion may be observed.

One strain (S 84) is a larger spirochete with irregular and rather loose coils, measuring 16 to 20 microns in length and 0.4 to 0.7 micron in width (*fig. 6*).



Darkfield photomicrographs:

FIG. 4. S 31–78 (Group C) ×1140.
FIG. 5. S 79 (Group C) ×1140.
FIG. 6. S 84 (Group B) ×1140.
FIG. 7. S 58–1 (Group A) ×1140.

In older cultures the motion is chiefly rotating whereas in young cultures an active serpentine motion of the flexible organism may be observed. Terminal filaments which are much thinner than the spirochete body and often slightly wavy have been seen repeatedly under the darkfield, but we have not been able to demonstrate them by staining methods.

Terminal filaments are also, but only rarely found in the spirochetes of the strains S 26, S 58–1 and S 58–33. The average length of these organisms is 20 to 25 microns, their diameter 0.8 to 1 micron (*fig. 7*). The central portion

of the spirochetes consists of regularly and closely wound coils, the distal coils being more open and often bent at an obtuse angle to the axis of the spirochete. In an actively motile culture the motion is rapid and exclusively rotating. Sluggishly motile organisms show the motility to be confined to the distal spirals, the center remaining motionless and rigid. A similar morphology and type of motility may be observed in some of the culture strains of *T. pallidum* (Reiter, Kasan).

MEDIA AND GROWTH REQUIREMENTS

All cultures grew only under anaerobic conditions. They were incubated in anaerobe jars with the exception of the cultures in Brewer's thioglycollate medium (30) which were not placed in the jars. The incubation time necessary to obtain satisfactory growth varied from 3 to 21 days. Subcultures from young, i.e. 5 to 7 day old cultures usually showed initial growth 3 to 5 days following transplantation. Subcultures from fluid media grew out faster than those from solid media. Parallel subcultures made in a liquid medium, a shake tube and a plate culture usually showed growth first in the liquid medium and last in the plate cultures. All cultures were incubated for 3 weeks before being discarded as negative.

The basis for all culture media with the exception of the thioglycollate medium was fresh beef heart infusion broth.

To 500 gm. of freshly ground beef heart was added 1,000 cc. of water. The mixture was infused overnight in the refrigerator, and on the following morning boiled for 10 minutes. The broth was filtered through filter paper. To each 1,000 cc. of broth were added 5 gm. sodium chloride and 10 gm. thio peptone (Wilson).

A limited number of cultures has been made in pork infusion broth and pork infusion agar. Spirochete growth has been obtained in these media, but we do not have enough data to compare the pork with the beef heart infusion media. The beef heart infusion was modified by the addition of various combinations of ascitic fluid, blood, agar, reducing agents and by varying the pH of the media, as shown in Table II.

The blood used was defibrinated rabbit blood. For blood agar plates 1 cc. of blood was added to 10 cc. of the medium. Four drops of blood were added to the liquid beef heart infusion media (Medium III to Va).

Not all lots of ascitic fluid were found suitable for the cultivation of mouth spirochetes. Specimens that were cloudy or contained bile were rejected. We have not been able to use determinations of the pH and the protein content as a guide in choosing a suitable ascitic fluid and have found it most helpful to test the ascitic fluid by using it for the cultivation of the Nichols and Noguchi strains of *T. pallidum*. If these 2 strains grew in the ascitic fluid in question, it could always be used for the cultivation of the mouth spirochetes. The ascitic fluid was heated at 56°C. for 1 hour. All ascitic fluid media contained ascitic fluid in a final concentration of 10 per cent.

Special care was taken to prepare clear, transparent agar media without

sediment, because the delicate young spirochete colonies are more easily visible in a clear medium and particles of a flocculent sediment may be confused with spirochete growth.

Sodium thioglycollate was added to the media before autoclaving, in a final concentration of 0.1 per cent.

The cysteine hydrochloride was prepared in a 1 per cent solution and sterilized by autoclaving it for 5 minutes at 15 lb. pressure. It was neutralized with N/1 sodium hydroxide just prior to its addition to the different media, to which enough cysteine hydrochloride was added to result in a final concentration of 0.1 per cent.

Media I and Ia were found to be satisfactory media for mixed cultures of spirochetes and bacteria, but they did not support the growth of pure cultures of mouth spirochetes in successive subcultures. The stimulating effect of contaminating microorganisms on spirochete growth has been emphasized by Vinzent and Seguin (10) and Kolmer and Kast (19), and we can confirm the observations of these authors by our own experience.

A pH of 7.2 to 7.9 is generally regarded as the optimum hydrogen ion concentration for spirochete media (31, 32, 34). Weiss and Wilkes-Weiss report that spirochetes (*T. pallidum*) do not develop at a pH lower than 6.9 and that they disintegrate in media of a pH of 6.1 to 6.4, whereas Scheff (33) was able to maintain viable spirochete cultures in media of pH of 6.2. We have made a number of parallel cultures in media with a pH of 6.4 to 6.6 and 7.3 to 7.5. Spirochete growth was obtained at either hydrogen ion concentration. In liquid media there was little or no difference in the amount of growth in the acid and alkaline media, whereas in solid media the alkaline medium was found to be definitely superior.

Media II and IIa. The addition of small amounts of agar to liquid media is known to be favorable for the cultivation of spirochetes (10, 32, 34). The addition of 0.05 per cent agar to Medium I and Ia resulted in slightly improved growth of all strains. However only 3 strains, S 26, S 58–1 and S 58–33 grow satisfactorily in these media in successive pure subcultures; all other strains grew poorly if at all after a limited number of pure subcultures.

Media IV and IVa. Spirochete growth in these media was better than in the corresponding ascitic fluid broths. Subcultures to the tenth generation have been carried on with a comparable amount of growth in the tenth and in the first subculture. Since all cultures were incubated in the anaerobe jar we omitted the addition of a reducing agent in a number of cultures.

Media III and IIIa. Satisfactory cultures and repeated subcultures were obtained in these media, but the results were not as uniformly good as in the corresponding media (Media IV and IVa) containing cysteine hydrochloride. See also Media VI, VIa, IX, IXa, XI, XIa.

Media V and Va. The growth in these media was better than in the blood broth without agar (Media IV, IVa) or in the corresponding ascitic fluid media (Media II, IIa). Spirochete growth as well as motility was definitely better in the alkaline medium, which may be used as a stock culture medium. The

cultures may remain viable for 4 to 6 weeks, but for the maintenance of stock strains it is advisable to make the transfers every 2 to 3 weeks.

Spirochete cultures in solid media were made in deep agar shake tubes and in plate cultures. All solid media containing blood were used in plates exclusively, whereas the ascitic fluid media were used in shake tubes or plates. Optimum conditions of agar concentration or pH were tested in the shake tubes rather than in the plates.

Medium VII was used in shake tubes and plates. It was used primarily for the isolation of cultures and was found suitable as a stock culture medium for only 3 strains, S 26, S 58–1 and S 58–33, the only 3 of our strains that will grow in successive cultures without the addition of blood to the media. For isolation purposes it was found to be a good and very reliable medium, particularly when it was used in shake tubes. When used in double layer plates (Medium XIII) results were satisfactory but not quite as dependable.

Medium VI. Spirochete growth in this medium was obtained. However the results were not as consistently good as in the corresponding medium containing cysteine hydrochloride (Medium VII). Similar effects of the omission of cysteine hydrochloride were noted in media with a higher agar or a higher hydrogen ion concentration: Media VIa, IX, IXa, XI, XIa.

Medium VIII. Growth of mouth spirochetes was frequently obtained, but not with the same regularity as in the comparable cysteine medium (Medium VII).

Media IX to XIIa. Spirochete growth has been obtained, however positive results have been observed with much less regularity than in the 1 per cent agar media (Media VII, VIIa).

Media VIa, VIIa, IXa, Xa, XIa, XIIa. Cultivation of spirochetes was found to be possible in these media, but we have frequently encountered negative results with positive controls in the more alkaline media (Media VI, VII, IX, X, XI, XII).

Medium XIV. Colonies of mouth spirochetes were obtained in these plates. The results were irregular and the growth was usually less abundant than in control shake tubes containing 1 per cent agar (Medium VII). Growth was not improved by the addition of cysteine hydrochloride or dextrose or both to the blood agar: *Media XV, XVI, XVII.*

Medium XVIII. Spirochete growth was more abundant and was obtained with much greater regularity than in any of the preceding blood agar media (Media XIV to XVII). In an effort to furnish the most favorable conditions for spirochete growth special attention was paid to the age of the blood which was used, to the age and type of culture from which the transfer was made, and to the size of the inoculum. The age of the blood (1 to 24 days) was found to be of no influence. Transfers from solid or from liquid media grew with equal regularity, but subcultures from liquid media appeared more advisable. The colonies were sometimes difficult to break up, which resulted in unevenly distributed growth or growth surrounding the inoculum only. The need for a large inoculum was especially evident in blood agar plates. We have as a rule

used as inocula: one 2 mm. loopful of an undiluted broth culture; one 2 mm. loopful of a broth culture dilution (3 drops of culture in 5 cc. of broth); one whole colony or one drop of a dilution of such colony in 5 cc. of broth. The age of the inoculum (5 to 28 days) did not seem to be decisive but was of definite influence. Stock cultures which are transferred regularly every 14 to 16 days will usually show initial growth after 3 to 5 days. Transfers from 5 to 7 day old stock cultures or comparable mixed broth cultures have given us the most favorable results in all media and were therefore used preferably for the inoculation of blood agar plates.

Stock cultures of mouth spirochetes may be kept in deep agar shake tubes (Medium VII), in Medium V or in Brewer's thioglycollate medium. Medium VII can be used only for the strains that do not require blood. We have found it most convenient to keep stock cultures in the thioglycollate medium even though transfers are necessary every two to three weeks, whereas cultures in both other media need be transferred only every 4 to 5 weeks.

The cultures in the thioglycollate medium were incubated at 37°C. but were not placed in anaerobe jars. One cc. of ascitic fluid or 4 to 5 drops of defibrinated rabbit blood were added to 12 cc. of thioglycollate medium. Three drops of culture were used as inoculum.

In the beginning of our work we lost a number of pure strains by keeping the stock cultures in Medium I, II, VII or thioglycollate medium with ascitic fluid. When it was found that the addition of blood rather than ascitic fluid would improve growth and viability, blood was added to the stock culture media, and we have been able to maintain all of our strains in media containing blood, a number of the strains having been carried on for as many as 30 generations. None of our strains has required the presence of fresh tissue. This raises the question as to whether blood may be used as a convenient substitute for fresh tissue. Three strains (S 26, S 58–1 and S 58–33) may be subcultured in media containing blood or ascitic fluid or serum. Sixteen strains grew in repeated subcultures only in media containing blood.

In an effort to determine which constituent of the blood contained the necessary growth factor, parallel cultures of these strains were made in thioglycollate medium plus defibrinated rabbit blood, serum, washed cells, laked blood, cell stroma or ascitic fluid. The results were as follows: All strains grew well on addition of whole blood (10 to 30 spirochetes per field). All strains grew on addition of laked blood or washed cells for at least six generations (5 to 10 spirochetes per field). All strains grew poorly (1 to 4 spirochetes per field) on addition of ascitic fluid or serum for one or two transfers, the third transfer always being negative. None of the strains grew on addition of cell stroma.

Eleven strains have been tested for their ability to attack certain carbohydrates. The medium used was yeast fermented beef heart infusion broth,[2] to which were added 10 per cent of ascitic fluid, 0.1 per cent of cysteine hydrochloride and 0.5 per cent of the various carbohydrates. The carbohydrates used were: dextrose, lactose, sucrose, maltose, raffinose, trehalose, salicin, inulin,

[2] The infusion was fermented by yeast before being made up into broth.

mannite, sorbitol, adonitol, inositol and dulcitol. The cultures were incubated for 2 weeks and pH determinations on them and on uninoculated control media were done. Three strains (S 26, S 58–1 and S 58–33) produced acid in the dextrose medium, causing a lowering of the pH of 0.3 to 1.5. The growth in the dextrose broth was not markedly better than in any of the other media. None of the other strains showed acid production in any of the media nor was there an increase of growth on addition of any of the sugars.

Serological studies have not been done by us. Eagle (35) has found 2 of our strains (S 26 and S 58–33) serologically related to each other and to culture strains of *T. pallidum* (Reiter, Kasan).

SUMMARY

Pure cultures of spirochetes have been obtained from fusospirochetal mouth lesions. The isolation of these strains was accomplished by filtration through Chamberland-Pasteur filters, by cultivation in deep agar shake tubes and by cultivation in the depth of blood agar plates.

Nineteen strains of mouth spirochetes may be divided into 3 groups on the basis of their morphology and their cultural characteristics:

Group A (S 26, S 58–1 and S 58–33): large, regularly coiled spirochetes which do not require blood for the maintenance of pure subcultures. These spirochetes ferment dextrose with the production of acid. Colonies in blood agar plates are surrounded by a zone of partial hemolysis.

Group B (S 84): irregularly coiled spirochetes which require the presence of blood for the maintenance of the strain. Their ability to attack carbohydrates has not yet been determined. These spirochetes have refused to grow in the depth of blood agar plates.

Group C (the remaining 15 strains): small, regularly coiled spirochetes which require the presence of blood in stock culture media. Eight spirochetes of this group do not ferment dextrose. The other 7 strains have not been studied sufficiently to report on their carbohydrate metabolism. Colonies in blood agar are surrounded by a zone of partial hemolysis.

Our appreciation is expressed to an anonymous donor who has made this work possible by funds generously granted through Dr. Louis D. Coriell; also to Dr. Coriell whose clinical experience with the treatment of periodontal infections initiated the research and whose interest supported it with encouragement and suggestions. To Dr. J. Howard Brown we are indebted for helpful consultation and adequate laboratory facilities; to Mrs. Lucille B. Robinson for valued assistance.

REFERENCES TO LITERATURE

1. Tunnicliff, R., *J. A. D. A.*, **26:** 1987, 1939.
2. Kline, B. S., *J. Infect. Dis.*, **32:** 481, 1923.
3. Rosebury, T. and Foley, G., *J. A. D. A.*, **26:** 1798, 1939.
4. Smith, D. T., *Oral Spirochetes and Related Organisms in Fuso-spirochetal Disease* (Baltimore, Williams & Wilkins Co.: 1932).
5. Smith, D. T., *J. Infect. Dis.*, **46:** 303, 1930.
6. Smith, D. T., *Am. Rev. Tuberc.*, **16:** 584, 1927.
7. Noguchi, H., *J. Exper. Med.*, **14:** 99, 1911.

8. Smith, D. T., *Arch. Surg.*, part II, **16:** 231, 1927.
9. Proske, H. O., and Sayers, R. R., *U. S. Public Health Rep.*, **49:** 1212, 1934.
10. Seguin, P., and Vinzent, R., *Ann. Inst. Pasteur*, **61:** 255, 1938.
11. Mühlens, P., *Deutsche med. Wchnschr.*, **32:** 797, 1906.
12. Mühlens, P., and Hartmann, M., *Ztschr. f. Hyg. u. Infektionskr.*, **55:** 84, 1906.
13. Noguchi, H., *J. Exper. Med.*, **16:** 194, 1912.
14. Rosebury, T., and Foley, G., *Proc. Soc. Exper. Biol. and Med.*, **47:** 368, 1941; *J. D. Res.*, **21:** 308, 1942.
15. Shmamine, T., *Zentralbl. f. Bakt.*, **65:** 311, 1912.
16. Noguchi, H., *J. Exper. Med.*, **15:** 81, 1912.
17. Vinzent, R., and Daufresne, M., *Compt. rend. Soc. de Biol.*, **116:** 490, 1934.
18. Proca, G., Danila, P., and Stroe, A., *Compt. rend. Soc. de Biol.*, **72:** 895, 1912.
19. Kolmer, J. A., and Kast, C. C., *Am. J. Syph., Gonor. and Ven. Dis.*, **6:** 1, 1940.
20. Robinson, G. H., *Am. J. Hyg.*, **3:** 483, 1923.
21. Ecker, E. E., and Weed, A. A., *J. Infect. Dis.*, **49:** 355, 1931.
22. Akatsu, S., *J. Exper. Med.*, **25:** 375, 1917.
23. Noguchi, H., in *The Newer Knowledge of Bacteriology and Immunology*, Jordan and Falk, editors (Chicago, Univ. of Chicago Press: 1928).
24. Muttermilch, S., and Seguin, P., *Compt. rend. Soc. de Biol.*, **88:** 28, 1923.
25. Proske, H. O., and Sayers, R. R., *U. S. Public Health Rep.*, **49:** 839, 1934.
26. Gates, F. L., *J. Exper. Med.*, **37:** 311, 1923.
27. Fortner, J., *Zentralbl. f. Bakt.*, I Orig., **108:** 155, 1928.
28. Fortner, J., *Zentralbl. f. Bakt.*, I Orig., **110:** 233, 1929.
29. Aksjanzew-Malkin, S., *Zentralbl. f. Bakt.*, **129:** 405, 1933.
30. Brewer, J. H., *J. A. M. A.*, **115:** 598, 1940.
31. Fukushima, B., *Scientific Reports of the Government Institute of Tokyo*, **6:** 533, 1927.
32. Weiss, C., and Wilkes-Weiss, D., *J. Infect. Dis.*, **34:** 212, 1924.
33. Scheff, G., *Zentralbl. f. Bakt.*, **35:** 134, 1935.
34. Kligler, I. J., and Robertson, O. H., *J. Exper. Med.*, **35:** 303, 1922.
35. Eagle, H., Personal Communication.

RELATIONSHIP OF THE GINGIVAL CREVICE AND THE ALVEOLAR CREST[1]

HENRY M. GOLDMAN, D.M.D.

Oral Pathology Laboratory, Harvard School of Dental Medicine, Boston, Mass.

Pathologic gingival crevices have been described by Gottlieb, Orban, Kronfeld, Box, and numerous other investigators. Their general concept is that the crest of the alveolar bone will undergo resorption if an inflammation in the gingiva persists for a long period of time. This is true in many cases. The present investigation, however, demonstrates that the inflammation of the gingiva does not cause, necessarily, resorption of the alveolar crest.

The study of serial microscopic sections of 30 human jaws reveals that neither the depth of the gingival crevice nor the degree of inflammatory cell infiltration in the subepithelial tissue are directly related to the condition of the alveolar crest. Sections showing shallow gingival crevices with small and large numbers of inflammatory cells in the gingiva may show either resorption of the alveolar crest or an apposition of bone. These processes may also be demonstrated under deep gingival crevices.

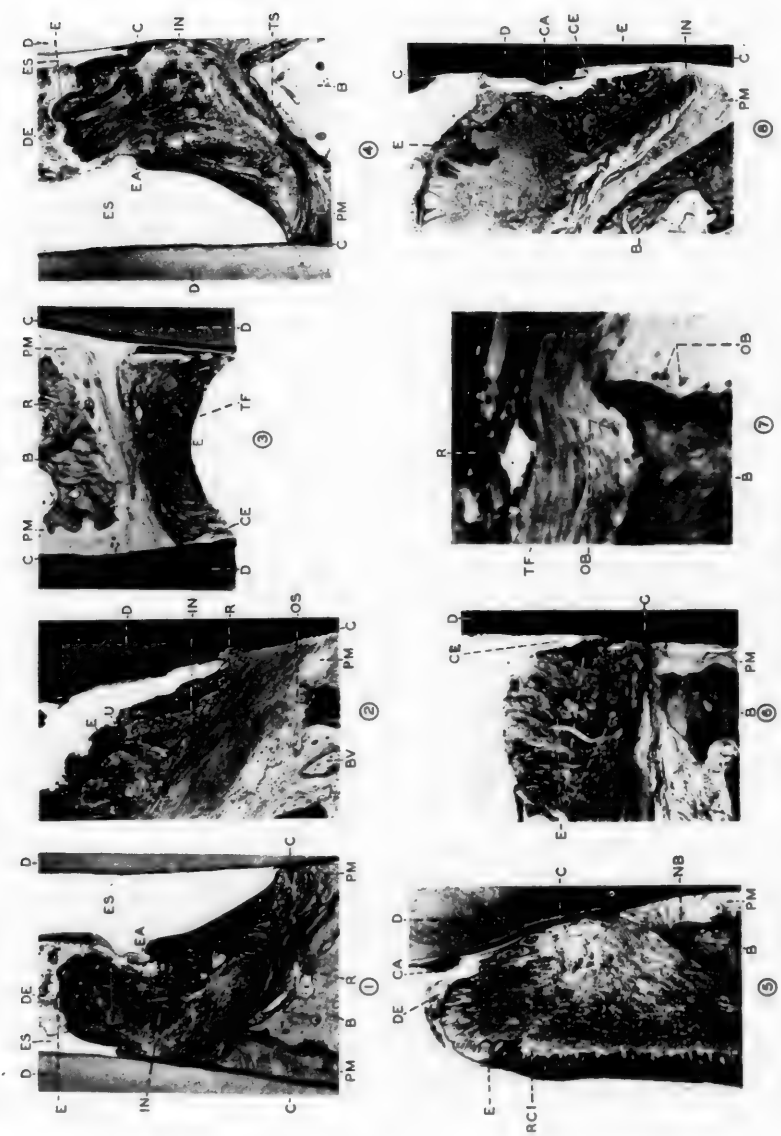
Evidence to support these statements may be found in the illustrations presented. The first 3 photomicrographs show resorption of the alveolar crest accompanying inflammation of the gingiva. The succeeding 4 photomicrographs demonstrate that under similar circumstances bone apposition may be seen. The last photomicrograph is of a section with a deep crevice, under which the bone seems inactive.

Fig. 1 is a photomicrograph of a mesiodistal section of the interdental tissues of a lower canine and premolar. The epithelial attachment is on the enamel. The depth of the crevices is comparable to that in *fig.* 4 and there is also a similar degree of inflammation in the gingiva. In this case resorption of the alveolar crest is evident in contrast to *fig.* 4 where apposition of bone is seen.

Fig. 2 is a photomicrograph of a mesiodistal section of a lower second premolar. The gingival crevice is deep and ulcerations of the epithelium may be seen. There is resorption of the cementum of the tooth beneath the epithelial attachment. Inflammation of the subepithelial tissue is evident. Osteoclastic resorption of the alveolar crest can be seen. This section may be compared with that shown in *fig.* 8.

Fig. 3 is a photomicrograph of a mesiodistal section between a maxillary canine and premolar. The epithelial attachments are on the cementum. The gingival crevices are comparatively shallow and a dense inflammatory infiltration can be seen in the subepithelial tissue. There is osteoclastic resorption

[1] Received for publication June 20, 1942. Revised by author Oct. 20, 1942.


D
ES
DE
EA
E
IN
C
PM
B
R
①
D
ES
DE
EA
C
PM
E
IN
C
PM
B
R
②
D
IN
R
OS
E
U
BV
PM
C
③
C
PM
B
R
PM
C
D
E
TF
CE
D
④
D
ES
DE
E
C
IN
TS
EA
ES
C
PM
D
B
⑤
DE
CA
D
C
NB
E
RCI
B
PM
⑥
CE
D
C
E
B
PM
⑦
R
OB
TF
OB
B
⑧
C
E
D
CA
CE
E
IN
B
PM
C

FIGS. 1–8

of the alveolar crest. This section should be compared with *fig.* 6 where apposition of bone may be seen, although conditions in the crevices seem similar.

Fig. 4 is a photomicrograph of a mesiodistal section of the interdental tissues of a lower canine and first premolar. The epithelial attachment is on the enamel. The gingival crevices are shallow. A marked infiltration of inflammatory cells can be seen in the subepithelial tissue. The transeptal fibers are intact. The crest of the alveolar bone shows a regular osteoblastic layer denoting a laying down of bone. Therefore, this section must be interpreted as an inflammation of the gingiva and an apposition of bone to the alveolar crest.

Fig. 5 is a photomicrograph of a buccolingual section of a lower first molar showing the buccal gingiva. In this section the epithelial attachment is on the cementum. Calculus can be seen lying in the shallow crevice. There is marked inflammation of the subepithelial tissue. The crest of the alveolar bone shows new bone formation. Although there has been recession of the gingival attachment and inflammation of the gingiva, apposition of bone to the alveolar crest is evident.

Fig. 6 is a low power photomicrograph of a mesiodistal section of the gingival crevice and alveolar bone of a lower premolar. Marked ulceration of the crevicular epithelium can be seen. There is also a pronounced inflammation of the subepithelial tissue. The transeptal fibers are intact. The significant bone alterations are shown in *fig.* 7 which is a high power photomicrograph of the alveolar crest shown in *fig.* 6. An osteoblastic layer surrounding the bone can be seen with evidence of new bone formation. Inflammatory cells are present directly above the transeptal fibers.

Fig. 8 is a photomicrograph of a mesiodistal section of a lower first molar with a deep crevice on the mesial aspect. Calculus can be seen lying in the pocket. The crevicular epithelium has grown into the subepithelial tissue in an anastomosing arrangement. The subepithelial tissue contains inflammatory cells. The alveolar bone appears inactive. There is no histologic evidence of resorption or new formation.

FIG. 1. D, dentin; C, cementum; ES, enamel space; DE, debris; E, epithelium; EA, bottom of crevice; IN, inflammatory cells; PM, periodontal membrane; B, bone; R, resorption.

FIG. 2. D, dentin; C, cementum; R, resorption; E, epithelium; U, ulceration; IN, inflammatory cells; BV, blood vessel; PM, periodontal membrane; B, bone; OS, osteoclast.

FIG. 3. D, dentin; C, cementum; CE, crevice; E, epithelium; IN, inflammatory cells; B, bone; R, resorption; PM, periodontal membrane.

FIG. 4. D, dentin; C, cementum; ES, enamel space; PM, periodontal membrane; B, bone; TS, transeptal fibers; DE, debris; E, epithelium; EA, bottom of crevice; In, inflammatory cells.

FIG. 5. D, dentin; C, cementum; CA, calculus; DE, debris; E, epithelium; RCI, inflammatory cells; B, bone; NB, new bone; PM, periodontal membrane.

FIG. 6. D, dentin; C, cementum; CE, crevice; E, epithelium; PM, periodontal membrane; B, bone.

FIG. 7. B, bone; OB, osteoblast; TF, transeptal fibers; R, inflammatory cells.

FIG. 8. D, dentin; C, cementum; CA, calculus; CF, crevice; E, epithelium; In, inflammatory cells; B, bone; PM, periodontal membrane.

CONCLUSIONS

The alveolar crest may show apposition of bone even in the presence of gingival inflammation and ulceration of the crevicular epithelium (*figs.* 4 to 7). In other cases under similar conditions resorption of the alveolar bone may be present (*figs.* 1 to 3). Therefore, it is concluded that the condition of the gingiva and of the gingival crevice does not necessarily determine whether or not there will be resorption of the alveolar crest, and that resorption may be influenced by other factors. Thus further evidence is presented to substantiate the clinical differentiation of two types of periodontal disease, gingivitis and marginal periodontitis.

REFERENCES

Box, H. K., *Studies in Periodontal Pathology* (Toronto, Univ. of Toronto Press; 1924).

Gottlieb, B., Etiology and Therapy of Alveolar Pyorrhea, *Ztschr. f. Stomatol.* **18:** 59, 1920.

Gottlieb, B. and Orban, B., *Biology and Pathology of the Tooth and Its Supporting Mechanism* (New York, Macmillan; 1938), p. 139.

Kronfeld, R., The Condition of the Alveolar Bone Underlying Periodontal Pockets, *J. Periodontol.* **6:** 22, 1935.

Weinmann, J. P., Progress of Gingival Inflammation into the Supporting Structures of the Teeth, *J. Periodontol.* **12:** 71, 1941.

Glickman, I. and Wood, H., Bone Histology in Periodontal Disease, *J. Dent. Research* **21:** 35, 1942.

SUPPLEMENT

CONTENTS

I. Sustaining subscribers to the Journal of Dental Research, 1942 565
II. International Association for Dental Research. Register of Members as of December 1, 1942, compiled by E. H. Hatton, General Secretary 566
III. Books received, 1942 578

I. Sustaining Subscribers to the Journal of Dental Research, 1942

Dr. J. L. T. Appleton, *Philadelphia, Pa.*
Dr. Robert E. Blackwell, *Chicago, Ill.*
Dr. O. W. Brandhorst, *St. Louis, Mo.*
Dr. Arthur E. Corby, *New York, N. Y.*
Dr. Charles G. Darlington, *New York, N. Y.*
Dr. M. Diamond, *New York, N. Y.*
Dr. Lewis E. Ford, *Los Angeles, Calif.*
Dr. J. T. Gore, *Philadelphia, Pa.*
Dr. Louis I. Grossman, *Philadelphia, Pa.*
Dr. Edward Hatton, *Chicago, Ill.*
Dr. Samuel Hemly, *New York, N. Y.*
Dr. Isadore Hirschfeld, *New York, N. Y.*
Dr. W. A. Jaquette, *Swarthmore, Pa.*
Dr. Paul C. Kitchin, *Columbus, Ohio*
Dr. Alfred L. Kohn, *New York, N. Y.*
Dr. Frances Krasnow, *New York, N. Y.*
Dr. John H. Longe, *Detroit, Mich.*
Dr. D. M. Milne, *Portland, Me.*
Dr. Celia Rich, *Nashville, Tenn.*
Dr. Roy J. Rinehart, *Kansas City, Mo.*
Dr. George J. Roth, *Philadelphia, Pa.*
Dr. Walter H. Scherer, *Houston, Texas*
Dr. Isaac Schour, *Chicago, Ill.*
Dr. Ernest G. Sloman, *San Francisco, Calif.*
Dr. Wilmer Souder, *Washington, D. C.*
First District Dental Soc., *New York, N. Y.*

II. International Association for Dental Research[1]

ROSTER OF SECTIONS[2, 3]

The Sections are listed and numbered in the order of their founding. In the following list of members the section numbers are used to indicate section membership.

1. New York
2. Boston
3. Chicago
4. Toronto
5. Ann Arbor
6. San Francisco
7. Minnesota
8. Philadelphia
9. Pittsburgh
10. St. Louis
11. Halifax
12. Vienna
13. New Haven (discontinued, 1940)
14. Cleveland
15. Winnipeg
16. London
17. Washington
18. Columbus
19. Louisville
20. Prague
21. Baltimore
22. Richmond
23. Rochester
24. Budapest
25. Chengtu
26. Johannesburg
27. Iowa City
28. Detroit
29. New Orleans
30. Palestine
31. Houston
32. Indianapolis

REGISTER OF MEMBERS

1. NORTH AMERICAN MEMBERS[4]

Myron S. Aisenberg, 20, Dental School, U. of Maryland, Baltimore, Md.
Frank Allen, Jr., 1844 Coles Bldg., Portsmouth, Ohio.
George M. Anderson, 20, 831 Park Ave., Baltimore, Md.
Bert G. Anderson, Medical School, Yale U., New Haven, Conn.
Edmund Applebaum, 1, 630 W. 168th St., New York, N. Y.
J. L. T. Appleton, 8, Dental School, U. of Pennsylvania, Philadelphia, Pa.
W. Harry Archer, 9, Dental School, U. of Pittsburgh, Pittsburgh, Pa.
S. S. Arnim, 22, Dental School, Medical College of Virginia, Richmond, Va.
Frances S. Arnold, 17, National Institute of Health, Bethesda, Md.
Wallace D. Armstrong, 7, Medical School, U. of Minnesota, Minneapolis, Minn.
H. E. Ash, 17, Army Medical Museum, Washington, D. C.
Harry H. Asher, 3, 55 E. Washington St., Chicago, Ill.

---

[1] The proceedings of the twentieth general meeting (New York, March 14–15, 1942) were published in the *J. D. Res.*, 20: 285, 1942 (this volume).

[2] *Sectional meetings in 1942* were held on the following schedules as reported by sectional secretaries. *Boston*—March 2, April 21. *Chicago*—October 9, November 13, December 11. *Minnesota*—April 15, October 23. *New York*—January 29, May 28. *Pittsburgh*—January 5, May 13, October 5. *St. Louis*—February 9, December 17. *Washington*—January 28, April 14, November 17.

[3] The following members represented their sections in the Council during 1942[4]: Philip Jay (5), M. S. Aisenberg (21), L. M. S. Miner (2), R. G. Agnew (25), A. G. Brodie (3), T. J. Hill (14), P. C. Lowery (28), V. D. Cheyne (32), E. Kotanyi (31), G. Van Huysen (19), J. T. O'Rourke (19), P. J. Brekhus (7), S. L. Tiblier (29), L. M. Waugh (1), T. J. Cook (8), J. L. T. Appleton (8), H. E. Friesell (9), Harry Bear (22), A. D. Brashear (22), Joseph Volker (23), Norman Simmons (23), H. B. McCauley (23), H. B. G. Robinson (10), W. E. Koch, Jr. (10), W. C. Fleming (6), A. J. McDonagh (15), A. D. Mason (4), H. T. Dean (17), F. S. Arnold (17), A. W. Bryan (27), J. H. Kaiser (18), H. J. Merkeley (15). Following sections did not participate in Council activities: Budapest, Halifax, Johannesburg, London, Palestine, Prague, Vienna. Dental Materials Group represented in the Council by W. S. Crowell.

[4] A number following a name indicates membership in a section as shown in the key to the sections immediately preceding this list.

SPENCER R. ATKINSON, 407 Professional Bldg., Pasadena, Cal.
LOUIE T. AUSTIN, 7, Mayo Clinic, Rochester, N. Y.

*LAWRENCE W. BAKER, 2, 230 Beacon St., Boston, Mass.
ZELMA BAKER, 3, 950 E. 59th St., Chicago, Ill.
J. STANLEY BAGNALL, 11, Dental School, Dalhousie U., Halifax, Nova Scotia.
RONALD BARBER, 17, 2319 Grant St., Berkeley, Calif.
HENRY A. BARTELS, 1, 57 W. 57th St., New York, N. Y.
WILLIAM BAUER, 10, 3556 Caroline St., St. Louis, Mo.
JOHN R. BEALL, 17, National Bureau of Standards, Washington, D. C.
HARRY BEAR, 22, Dental School, Medical College of Virginia, Richmond, Va.
GEORGE L. BEAN, 6, 5 Fitzhugh Bldg., San Francisco, Calif.
RAYMOND J. BEAN, 11, Medical School, Dalhousie U., Halifax, Nova Scotia.
HERMANN BECKS, 6, Hooper Foundation, U. of California, San Francisco, Calif.
MILTON C. BERMAN, Camp Grant, Rockford, Ill.
*ADOLPH BERGER, 1, 10 E. 74th St., New York, N. Y.
OLAF BERGHEIM, 3, 1817 West Polk St., Chicago, Ill.
JOSEPH L. BERNIER, 17, Hospital Center #15, Camp Barkley, Tex.
ELMER S. BEST, 7, 801 Medical Arts Bldg., Minneapolis, Minn.
FRANK E. BEUBE, 1, 630 West 168th St., New York, N. Y.
GERRIT BEVELANDER, 1, 477 First Ave., New York, N. Y.
B. G. BIBBY, 2, Dental School, Tufts College, Boston, Mass.
E. ROY BIER, 15, 7–8 Medical Arts Bldg., Winnipeg, Canada.
S. N. BLACKBERG, 3, 332 S. Michigan Ave., Chicago, Ill.
R. E. BLACKWELL, 3, 180 N. Michigan Ave., Chicago, Ill.
JAMES R. BLAYNEY, 3, 950 East 59th St., Chicago, Ill.
ROSS F. BLEIKER, 10, 333 Metropolitan Bldg., St. Louis, Mo.
DAVID D. BLOOM, 2, 412 Beacon St., Boston, Mass.
*THEODOR BLUM, 1, 101 East 79th St., New York, N. Y.
*FRED R. BLUMENTHAL, 2, 475 Commonwealth Ave., Boston, Mass.
CHARLES F. BODECKER, 1, 630 West 168th St., New York, N. Y.
L. R. BOLING, 10, 4559 Scott Ave., St. Louis, Mo.
WALTER A. BOSSERT, 1, 1346 Taylor Ave., New York, N. Y.
CARL O. BOUCHER, 18, Dental School, Ohio State U., Columbus, Ohio.
EARL P. BOULGER, 3, 27 S. Pulaski St., Chicago, Ill.
HAROLD K. BOX, 4, 86 Bloor St., Toronto, Canada.
PAUL E. BOYLE, 2, Dental School, Harvard U., Boston, Mass.
SIGMUND F. BRADEL, 3, 950 East 59th St., Chicago, Ill.
EWING P. BRADY, 10, 7239 Northmoor Drive, St. Louis, Mo.
J. C. BRAUER, 27, Dental School, Iowa State U., Iowa City, Iowa.
THURLOW W. BRAND, 9, Dental School, U. of Pittsburgh, Pittsburgh, Pa.
OTTO W. BRANDHORST, 10, 4952 Maryland Ave., St. Louis, Mo.
ALTON D. BRASHEAR, 22, Dental School, Medical College of Virginia, Richmond, Va. (with U. S. Army)
ROBERT E. BRAWLEY, 2301 Salem Ave., Dayton, Ohio.
CARL BREITNER, 1, 200 Cabrini Blvd., New York, N. Y.
PETER BREKHUS, 7, 1967 E. River Road, Minneapolis, Minn.
WALTER C. BRIDGE, 138 Algonquin Road, Chestnut Hill, Mass.
B. HOLLY BROADBENT, 14, 1400 Keith Bldg., Cleveland, Ohio.
ALLEN G. BRODIE, 3, 30 N. Michigan Ave., Chicago, Ill.
RALPH H. BRODSKY, 1, 205 West 57th St., New York, N. Y.
A. J. BROUGHTON, 4, 86 Bloor St., Toronto, Canada.

* Charter member.

HOMER C. BROWN, 1816 Franklin Ave., Columbus, Ohio.
J. B. BROWN, 18, Hamilton Hall, Ohio State U., Columbus, Ohio.
J. C. BROWN, 192 Main St., Andover, Mass.
ROBERT K. BROWN, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
A. W. BRYAN, 27, Dental School, Iowa State U., Iowa City, Iowa.
MAURICE BUCHBINDER, 1, 150 West 55th St., New York, N. Y.
R. W. BUNTING, Dental School, U. of Michigan, Ann Arbor, Mich.
LESTER W. BURKET, 8, Dental School, U. of Pennsylvania, Philadelphia, Pa.
CASPER G. BURN, Long Island Hospital Medical School, 350 Henry St., Brooklyn, New York.
PHILIP BURWASSER, 3, 950 East 59th St., Chicago, Ill.
ALDEN J. BUSH, 150 Broad St., Columbus, Ohio.

LESTER R. CAHN, 1, 944 Park Ave., New York, N. Y.
C. WILLARD CAMALIER, 17, 1726 Eye St., N. W., Washington, D. C.
CLARENCE P. CANBY, 17, Starke General Hospital, Charlestown, S. C.
MALCOLM W. CARR, 1, 52 East of 61st St., New York, N. Y.
H. J. CAUL, 17, 812 Jefferson St., Washington, D. C.
SAMUEL W. CHASE, 14, 2109 Adelbert Road, Cleveland, Ohio.
VIRGIL D. CHEYNE, 32, Dental School, U. of Indiana, Indianapolis, Ind.
KERMIT C. CHRISTENSEN, Dental School, St. Louis U., St. Louis, Mo.
H. R. CHURCHILL, 8, Dental School, U. of Pennsylvania, Philadelphia, Pa.
ROLAND S. CLAFLIN, 3, 3724 Fullerton Ave., Chicago, Ill.
*GEORGE W. CLAPP, 1, 220 West 42nd St., New York, N. Y.
STANLEY W. CLARK, 3, 311 E. Chicago Ave., Chicago, Ill.
OLIVER W. CLOUGH, 22, Dental School, Medical College of Virginia, Richmond, Va.
JOSEPH R. COHEN, 7, 1449 Medical Arts Bldg., Minneapolis, Minn.
THOMAS J. COOK, 8, Dental School, U. of Pennsylvania, Philadelphia, Pa.
RICHARD H. COLEMAN, 71 Elm St., Hartford, Conn.
S. D. COLLINS, 17, National Institute of Health, Bethesda, Md.
EDGAR E. COOLIDGE, 3, 25 E. Washington St., Chicago, Ill.
H. V. COTTRELL, 18, 404 Hartman Bldg., Columbus, Ohio.
THOMAS COWLINGS, 4, 1 Grant St., Toronto, Canada.
GERALD J. COX, 9, Mellon Institute, U. of Pittsburgh, Pittsburgh, Pa.
HERBERT D. COY, 22, Baker & Co., Newark, N. J.
ARTHUR B. CRANE, 17, 1726 Eye St., N. W., Washington, D. C.
WILLIAM H. CRAWFORD, 32, Dental School, U. of Indiana, Indianapolis, Ind.
KENT H. CROSS, 2355 Evans Ave., Denver, Colo.
WALTER S. CROWELL, 8, 211 S. 12th St., Philadelphia, Pa.
MARY C. CROWLEY, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
FRANK R. CUSHMAN, 2, 12 Bay State Road, Boston, Mass.

A. A. DAHLBERG, 3, 122 S. Michigan Ave., Chicago, Ill.
FRANCIS H. DALEY, 2, 1085 Boylston St., Boston, Mass.
CHARLES G. DARLINGTON, 1, 209 E. 23rd St., New York, N. Y.
WILBUR M. DAVIS, 401 Exchange Bldg., Orlando, Florida.
M. L. DEAKINS, 2, Dental School, Harvard U., Boston, Mass.
H. TRENDLEY DEAN, 17, National Institute of Health, Bethesda, Md.
CHARLES F. DEATHERAGE, Dept. of Public Health, Springfield, Ill.
GEORGE B. DENTON, 3, 311 E. Chicago Ave., Chicago, Ill.
JOHN A. DETLEFSON, 215 Lafayette Ave., Swarthmore, Pa.
MOSES DIAMOND, 1, 55 Central Park West, New York, N. Y.

---

* Charter member.

G. B. Diefenbach, 19, 129 E. Broadway, Louisville, Ky.
Merle C. Dixon, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
Edward C. Dobbs, 21, Dental School, U. of Maryland, Baltimore, Md.
W. Gershom Downs, Jr., Last address—New York, N. Y.
Robert P. Dressel, 2165 Adelbert Road, Cleveland, Ohio.
*H. S. Dunning, 1, 33 E. 68th St., New York, N. Y.
William B. Dunning, 1, 140 E. 80th St., New York, N. Y.
H. Shirley Dwyer, 1, 80 Hanson Place, Brooklyn, N. Y.

Kenneth R. Easlick, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
B. R. East, 1, 600 West 168th St., New York, N. Y.
George S. Easton, 27, Dental School, Iowa State U., Iowa City, Iowa.
Hugh M. Eaton, 11, Birks Bldg., Halifax, Nova Scotia.
Linden F. Edwards, 18, Anatomy Dept., Ohio State U., Columbus, Ohio.
Ralph Edwards, 1108 East 10th St., Kansas City, Mo.
William E. Ehrich, 8, Medical School, U. of Pennsylvania, Philadelphia, Pa.
Frederick C. Elliott, 31, Texas Dental College, Houston, Texas.
M. D. Elliott, 2, 358 Commonwealth Ave., Boston, Mass.
A. W. Ellis, 4, 80 King St. West, Toronto, Canada.
Elias Elvolve, 17, National Institute of Health, Bethesda, Md.
Leroy M. Ennis, 8, 4001 Spruce St., Philadelphia, Pa.
John Enright, 9, 6600 Dalzell St., Pittsburgh, Pa.
Irwin A. Epstein, 7, 357 Lowry Medical Arts Bldg., St. Paul, Minn.
Sidney Epstein, 6, 516 Sutter St., San Francisco, Calif.
B. Edwin Erickson, 17, 3726 Connecticut Ave., N. W., Washington, D. C.

Leigh C. Fairbank, 17, 1726 Eye St., N. W., Washington, D. C.
Philip S. Faillo, 3, 1908 St. Charles Road, Maywood, Ill.
Alden W. Faulkner, 11, 69 Gottingen St., Halifax, Nova Scotia.
A. H. Fee, 22, Dental School, Medical College of Virginia, Richmond, Va.
William L. Fickes, 9, Dental School, U. of Pittsburgh, Pittsburgh, Pa.
W. A. Figg, 3, 417 Morris Bldg., Joliet, Ill.
Sidney B. Finn, 23, 90 Colby St., Rochester, N. Y.
Charlotte Fisk, 7, 322 Eighteenth Ave., S. E., Minneapolis, Minn.
Willard C. Fleming, Dental School, U. of California, San Francisco, Calif.
John C. Forbes, 22, Dept. of Chemistry, Medical College of Virginia, Richmond, Va.
Leonard S. Fosdick, 3, 311 E. Chicago Ave., Chicago, Ill.
Lewis Fox, 1, 85 Washington St., South Norwalk, Conn.
H. T. Fraser, 17, National Institute of Health, Bethesda, Md.
Charles W. Freeman, 3, 8 S. Michigan Ave., Chicago, Ill.
F. C. Friesell, 9, Dental School, U. of Pittsburgh, Pittsburgh, Pa.
H. E. Friesell, 9, Dental School, U. of Pittsburgh, Pittsburgh, Pa,
B. John Frey, 3, 4368 Milwaukee Ave., Chicago, Ill.

A. H. Gabel, 8, Dental School, U. of Pennsylvania, Philadelphia, Pa.
William Gafafer, 17, National Institute of Health, Division of Industrial Hygiene, Bethesda, Md.
Boyd S. Gardner, 7, Mayo Clinic, Rochester, Minn.
Leland H. Garrison, 10, Fairfield, Ill.
Mathew H. Garvin, 15, 314 Sommerset Bldg., Winnipeg, Canada.
*William J. Gies, 1, 632 West 168th St., New York, N. Y.
Raleigh Gilchrist, 17, National Bureau of Standards, Washington, D. C.

* Charter member.

C. C. Gilkison, 14, 10465 Carnegie Ave., Cleveland, Ohio.
J. Raymond Gill, 6, 450 Sutter St., San Francisco, Calif.
* H. W. Gillett, 140 57th St., New York, N. Y.
J. T. Ginn, 23, 260 Crittenden Blvd., Rochester, N. Y.
Irving Glickman, 2, Dental School, Tufts College, Boston, Mass.
Maurice A. Goldberg, 17, 1726 Eye St., N. W., Washington, D. C.
Henry M. Goldman, 2, Dental School, Harvard U., Boston, Mass.
Harry Goldstein, 21, 2408 Place, Baltimore, Md.
J. Orton Goodsell, 5, 501 Second National Bank Bldg., Ann Arbor, Mich.
Samuel M. Gordon, 84–40 101st St., Richmond Hill, N. Y. (With U. S. Army.)
J. T. Gore, 8, 1737 Chestnut St., Philadelphia, Pa.
B. Gottlieb, Dental School, Baylor U., Dallas, Texas.
W. C. Graham, 18, Dental School, Ohio State U., Columbus, Ohio.
William K. Gregory, 1, American Museum of Natural History, New York, N. Y.
George W. Grieve, 4, 12 Bloor St., Toronto, Canada.
Louis I. Grossman, 8, Medical Arts Bldg., Philadelphia, Pa.
Webb B. Gurley, 10, 4559 Scott Ave., St. Louis, Mo. (With U. S. Army.)

Faith P. Hadley, 9, Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh, Pa
William H. Hahn, 21, Dental School, U. of Maryland, Baltimore, Md.
J. Frank Hall, Dental School, U. of Indiana, Indianapolis, Ind.
Carolyn Hammond, 3, 1757 West Harrison St., Chicago, Ill.
Edward Hampp, 17, National Institute of Health, Bethesda, Md.
Harold L. Hansen, 33 Riverside Ave., Rensselaer, N. Y.
R. W. Harrison, 3, 950 E. 59th St., Chicago, Ill.
Leroy P. Hartley, Ft. Winfield Scott.
Harry E. Harvey, Brooklyn, N. Y., U. S. Navy Dental Corps.
Charles E. Hatch, 3, 808 S. Wood St., Chicago, Ill.
Edward H. Hatton, 3, 311 E. Chicago Ave., Chicago, Ill.
A. Borden Haverstock, 11, Roy Bldg., Halifax, Nova Scotia.
James C. Healy, Medical School, Tufts College, Boston, Mass.
* Milo Hellman, 1, 57 West 57th St., New York, N. Y.
Samuel Hemley, 1, 209 E. 23rd St., New York, N. Y.
Elizabeth S. Hemmens, 3, Bacteriology Dept., U. of Chicago, Chicago, Ill.
Fred Herzberg, 3, 808 S. Wood St., Chicago, Ill. In Service.
Peter Hidnert, 17, National Bureau of Standards, Washington, D. C.
Lester B. Higley, 27, 705 Summit Ave., Iowa City, Iowa.
Thomas J. Hill, 14, 2085 Adelbert Road, Cleveland, Ohio.
Maynard K. Hine, 3, 808 S. Wood St., Chicago, Ill.
I. Hirschfeld, 1, 730 Fifth Ave., New York, N. Y.
Harold C. Hodge, 23, Medical-dental School. U. of Rochester, Rochester, N. Y.
M. M. Hoffman, 3, 3210 Potomac Ave., Chicago, Ill.
Franklin Hollander, 1, Mt. Sinai Hospital, 101st St. and Fifth Ave., New York, N. Y.
George M. Hollenbeck, 6, Medico-dental Bldg., Los Angeles, Calif.
Houghton Holliday, 1, 630 West 168th St., New York, N. Y.
W. B. Holmes, 137 N. Adams St., Fremont, Ohio.
B. L. Hooper, Dental School, U. of Nebraska, Lincoln, Neb.
Ernest A. Hooton, 2, Peabody Museum, Harvard U., Boston, Mass.
* Percy R. Howe, 2, Forsythe Dental Infirmary, Boston, Mass.
A. O. Hubbell, 7, Mayo Clinic, Rochester, Minn.
George A. Hughes, 6, Medical Bldg., Oakland, Calif.
F. C. Husband, 4, 2 Bloor St., Toronto, Canada.

---

* Charter member.

* R. G. Hutchinson, Jr., 1, Rensselaer Road, Essex Falls, N. J.
* Thaddeus P. Hyatt, 1, Wee Haven, Davenport Ridge Road, Stamford, Conn.

Robert H. Ivy, 8, Dental School, U. of Pennsylvania, Philadelphia, Pa.

Lawrence H. James, 17, Bacteriology Dept., U. of Maryland, College Park, Md.
E. A. Jasper, School of Dentistry, U. of Tennessee, Memphis, Tenn.
Philip Jay, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
Paul H. Jeserich, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
L. R. Johnson, 3, 311 E. Chicago Ave., Chicago, Ill.
Martha R. Jones, 29 Hill Point Ave., San Francisco, Calif.
Luzerne G. Jordan, 17, 1801 Eye St., N. W., Washington, D. C.
Brooks Juett, 19, 1112 First National Bnk. Bldg., Louisville, Ky.
Ellis B. Jump, 3, 950 East 59th St., Chicago, Ill.

J. H. Kaiser, 18, 1616 Main St., Columbus, Ohio.
Theodore Kaletsky, 1, 101 East 79th St., New York, N. Y.
Oscar Kanner, 3, Hastings State Hospital, Ingleside, Neb.
F. F. Kanthak, 3, Lawson General Hospital, Atlanta, Ga.
Harry Kaplan, 17, Farragut Medical Bldg., Washington, D. C.
Maxwell Karshan, 1, 632 West 168th St., New York, N. Y.
* V. H. Kazanjian, 2, 475 Commonwealth Ave., Boston, Mass.
Robert Keilty, 17, Connecticut Apartments, Washington, D. C.
Harry E. Kelsey, 21, 835 Park Ave., Baltimore, Md.
Alexander Ker, 6, 490 Post St., San Francisco, Calif.
William R. Kern, 3, 311 E. Chicago Ave., Chicago, Ill.
D. A. Kerr, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
Robert G. Kesel, 3, 808 S. Wood St., Chicago, Ill.
Edgar H. Keys, 10, University Club Bldg., St. Louis, Mo.
H. D. Kimball, 28, 910 Chalmers Ave., Detroit, Mich.
Thomas B. King, 1, 33 West 42nd St., New York, N. Y.
Richard H. Kingery, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
Paul C. Kitchin, 18, Dental School, U. of Ohio, Columbus, Ohio.
Arthur O. Klaffenbach, 27, Dental School, Iowa State U., Iowa City, Iowa.
Meyer Klatsky, 1, 401 Broadway, New York, N. Y.
Henry Klein, 17, National Institute of Health, Bethesda, Md.
A. H. Kniessner, 14, 2085 Adelbert Road, Cleveland, Ohio.
Holmes T. Knighton, 19, Medical School, U. of Louisville, Louisville, Ky.
John W. Knutson, 17, National Institute of Health, Bethesda, Md.
W. F. Koss, 32, Dental School, U. of Indiana, Indianapolis, Ind.
Harold W. Krogh, 17, 1835 Eye St., N. W., Washington, D. C.
W. E. Koch, Jr., 10, 4559 Scott Ave., St. Louis, Mo.
Emmerich Kotanyi, 31, Texas Dental College, Houston, Texas.
Frances Krasnow, 1, 422 East 72nd St., New York, N. Y.

Paul Lalonde, 23, 277 Alexander St., Rochester, N. Y.
R. A. Lasater, 3, 636 Church St., Evanston, Ill.
William F. Lasby, 7, Dental School, U. of Minnesota, Minneapolis, Minn.
Philip A. Leavitt, 2, 520 Beacon St., Boston, Mass.
William Lefkowitz, 1, 33 West 42nd St., New York, N. Y.
R. Wood Leigh, Connecticut Apartments, Washington, D. C.
Harold J. Leonard, 1, 630 West 168th St., New York, N. Y.

---

* Charter member.

VICTOR E. LEVINE, Medical School, Creighton U., Omaha, Neb.
SAMUEL J. LEWIS, 28, David Whitney Bldg., Detroit, Mich.
B. E. LISCHER, 10, 4559 Scott Ave., St. Louis, Mo.
ARTHUR P. LITTLE, 22, Dental School, Medical College of Virginia, Richmond, Va.
R. A. LOGAN, 22, Dental School, Medical College of Virginia, Richmond, Va.
WILLIAM H. G. LOGAN, 3, 1757 W. Harrison St., Chicago, Ill.
PAUL K. LOSCH, 2, Dental School, Harvard U., Boston, Mass.
P. C. LOWERY, 28, Lowery Bldg., Detroit, Mich.
RAYMOND T. LOWRY, 17, Naval Air Station, Norfolk, Va.
A. B. LUCKHARDT, 3, 950 East 59th St., Chicago, Ill.
G. R. LUNDQUIST, 3, 311 E. Chicago Ave., Chicago, Ill.
DANIEL F. LYNCH, 17, 1149 Sixteenth St., N. W., Washington, D. C.
HARRY LYONS, 22, 306 Professional Bldg., Richmond, Va.

EWING C. MCBEATH, 1, 630 West 168th St., New York, N. Y.
THOMAS F. MCBRIDE, 9, Dental School, U. of Pittsburgh, Pittsburgh, Pa.
JOHN OPPIE MCCALL, 1, 422 E. 72nd St., New York, N. Y.
FRANCIS P. MCCARTHY, 371 Commonwealth Ave., Boston, Mass.
H. B. MCCAULEY, 23, 260 Crittenden Blvd., Rochester, N. Y.
JESSE F. MCCLENDON, Hahnemann Hospital and Medical School, Philadelphia, Pa.
FRANK J. MCCLURE, 17, National Institute of Health, Bethesda, Md.
E. V. MCCOLLUM, 21, 615 N. Wolfe St., Baltimore, Md.
MARION W. MCCREA, 21, Dental School, U. of Maryland, Baltimore, Md. (With U. S. Army.)
ROBERT D. MCFARLAND, 18, 3391 High St., Columbus, Ohio.
FREDERIC S. MCKAY, 712 Exchange Bank Bldg., Colorado Springs, Colo.
HUGH W. MCMILLAN, 18, 1005 Union Central Bldg., Cincinnati, Ohio.
L. R. MAIN, 10, Metropolitan Bldg., St. Louis, Mo.
DONALD MAINLAND, 11, Medical School, Dalhousie, U., Halifax, Nova Scotia.
HOWARD MAJERISON, 3, 808 S. Wood St., Chicago, Ill.
RICHARD S. MANLEY, 6500 Meis Ave., North College Hill, Ohio.
ARVIN W. MANN, 14, 201 East 102nd St., Cleveland, Ohio.
JAMES B. MANN, 17, Brooks General Hosp., Ft. Sam Houston, Tex.
H. I. MARGOLIS, 2, 311 Commonwealth Ave., Boston, Mass.
ARNOLD D. MASON, 4, Faculty of Dentistry, Toronto U., Toronto, Canada.
MAURY MASSLER, 3, 808 S. Wood St., Chicago, Ill.
EDWIN H. MAUK, 6, Dental School, U. of California, San Francisco, Calif.
STERLING V. MEAD, 17, 1149 Sixteenth St., N. W., Washington, D. C.
EMANUEL G. MEISEL, 9, 121 University Place, Pittsburgh, Pa.
HOWARD J. MERKELEY, 15, 611 Medical Arts Bldg., Winnipeg, Canada.
* ARTHUR H. MERRITT, 1, 580 Fifth Ave., New York, N. Y.
JOHN V. MERSHON, 8, 1520 Spruce St., Philadelphia, Pa.
FREDERICK S. MEYER, 812 Medical Arts Bldg., Minneapolis, Minn.
ALBERT MIDGLEY, 1108 Union Trust Bldg., Providence, R. I.
GUY S. MILLBERRY, 6, R. F. D., Route #2, Box 181, Los Gatos, Calif.
B. F. MILLER, 3, 950 East 59th St., Chicago, Ill.
E. G. MILLER, JR., 1, 632 West 168th St., New York, N. Y.
HENRY A. MILLER, 8, 1930 Chestnut St., Philadelphia, Pa.
SAMUEL C. MILLER, 1, 57 West 57th St., New York, N. Y.
* L. M. S. MINER, 2, Dental School, Harvard U., Boston, Mass.
GEORGE R. MOORE, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
MARY MOORE, 8, 1614 Locust St., Philadelphia, Pa.
THOMAS E. MOORE, Ransom & Randolph, Toledo, Ohio.

* Charter member.

ARTHUR L. MORSE, 2, 29 Commonwealth Ave., Boston, Mass.
FRED W. MORSE, JR., 2, Medical School, Harvard U., Boston, Mass.
AUGUSTUS H. MUELLER, 3, 30 N. Michigan Ave., Chicago, Ill.
EMIL MUELLER, 3, 180 N. Michigan Ave., Chicago, Ill.
RAYMOND E. MYERS, 19, Dental School, U. of Louisville, Louisville, Ky.
VICTOR C. MYERS, 14, 2109 Adelbert Road, Cleveland, Ohio.

RAYMOND E. NAGLE, 2, 358 Commonwealth Ave., Boston, Mass.
C. T. NELSON, 2, 25 Shattuck St., Boston, Mass.
EGON NEUSTADT, 1, 133 East 58th St., New York, N. Y.
ISAAC NEUWIRTH, 1, 209 E. 23rd St., New York, N. Y.
ALLEN T. NEWMAN, 1, 209 E. 23rd St., New York, N. Y.
ANNA C. NICHOLS, 5333 Vine St., Philadelphia, Pa.
* FREDERICK B. NOYES, 3, 55 E. Washington St., Chicago, Ill.
HAROLD J. NOYES, 3, 30 N. Michigan Ave., Chicago, Ill.
VICTOR T. NYLANDER, 3, 919 N. Michigan Ave., Chicago, Ill.

JOHN S. OARTEL, 9, 7 Scenery Road, Pittsburgh, Pa.
EDITH OBLATT, 1, 27 Durham Road, White Plains, N. Y.
N. C. OCHSENHIRT, 9, Jenkins Arcade, Pittsburgh, Pa.
OREN A. OLIVER, 1101–1102 Medical Arts Bldg., Nashville, Tenn.
ALVIN J. OPPENHEIM, 1946 Oxford St., Pasadena, Cal.
B. ORBAN, 3, 1757 W. Harrison St., Chicago, Ill.
JOHN T. O'ROURKE, 19, Dental School, U. of Louisville, Louisville, Ky.
ELBERT B. OWEN, 10, 906 Olive St., St. Louis, Mo.
WARREN C. OXNER, 11, Birks Bldg., Halifax, Nova Scotia.

GEORGE C. PAFFENBARGER, 17, National Bureau of Standards, Washington, D. C. (With U. S. Navy.)
* BISSELL B. PALMER, 1, 667 Madison Ave., New York, N. Y.
CARROLL E. PALMER, 17, National Institute of Health, Bethesda, Md.
E. W. PAUL, 4, 2 College St., Toronto, Canada.
ELBERT C. PENDLETON, 3, 1757 W. Harrison St., Chicago, Ill.
ST. J. PERROTT, 17, National Institute of Health, Bethesda, Md.
HAROLD W. PETERSON, 29, 6363 St. Charles Ave., New Orleans, La.
LYLE S. PETTIT, 18, Dental School, Ohio State U., Columbus, Ohio.
FLOYD A. PEYTON, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
KENNETH R. PFEIFFER, 19, Dental School, U. of Louisville, Louisville, Ky.
RALPH W. PHILLIPS, 32, 1121 West Michigan St., Indianapolis, Ind.
MATHEW PODOLIN, 560 Delaware Ave., Buffalo, N. Y.
SAMUEL E. POND, 2, Marine Biological Lab., Woods Hole, Mass.
WESTON A. PRICE, 14, 8926 Euclid Ave., Cleveland, Ohio.
HERMAN PRINZ, 8, Dental School, U. of Pennsylvania, Philadelphia, Pa.
T. E. PURCELL, 10, 3556 Caroline St., St. Louis, Mo.

SAMUEL RABKIN, 711 Doctors Bldg., Cincinnati, Ohio.
DOROTHEA F. RADUSCH, 7, Dental School, U. of Minnesota, Minneapolis, Minn.
SAMUEL J. RANDALL, 18, Ft. Hayes, Columbus, Ohio.
WILLIAM M. RANDALL, Dental School, U. of Louisville, Louisville, Ky.
HOWARD E. RAPER, 10, Albuquerque, New Mexico.
KENNETH W. RAY, 7, 27 E. Fourth St., Minneapolis, Minn.
GERALD J. REED, 3, 1757 W. Harrison St., Chicago, Ill.

---

* Charter member.

JOSEPH S. RESTARSKI, 3, U. S. Navy.
W. J. RILEY, 15, 502 Medical Arts Bldg., Winnipeg, Canada.
STEPHEN G. RITCHIE, 11, 36 South St., Halifax, Nova Scotia.
LESLIE F. RITTERSHOFER, 5, 1103 S. University Ave., Ann Arbor, Mich.
HAMILTON B. G. ROBINSON, 10, 4559 Scott Ave., St. Louis, Mo.
J. BEN ROBINSON, 20, Dental School, U. of Maryland, Baltimore, Md.
* ALFRED P. ROGERS, 2, 60 Charlesgate, Boston, Mass.
WILLIAM M. ROGERS, 1, 630 West 168th St., New York, N. Y.
THEODOR ROSEBURY, 1, 632 West 168th St., New York, N. Y.
S. LEONARD ROSENTHAL, 8, Medical Arts Bldg., Philadelphia, Pa.
ARNE F. ROMNES, 3, 311 E. Chicago Ave., Chicago, Ill.
CHARLES E. RUDOLPH, 7, 909 Metropolitan Bldg., Minneapolis, Minn.
ROBERT W. RULE, 6, Dental School, U. of California, San Francisco, Calif.
WILLIAM B. RYDER, JR., 6, 2000 Van Ness Ave., San Francisco, Calif.

IRVING SALMAN, 1, 1188 Grand Concourse, New York, N. Y.
J. A. SALZMANN, 1, 654 Madison Ave., New York, N. Y.
B. G. SARNAT, 3, 808 S. Wood St., Chicago, Ill.
M. I. SCHAMBERG, 1, 20 East 57th St., New York, N. Y.
WALTER H. SCHERER, 31, 1620 Medical Arts Bldg., Houston, Texas.
C. H. SCHEU, 14, 14805 Detroit Ave., Cleveland, Ohio.
CARL A. SCHLACK, 17, U. S. Navy Dental School, Bethesda, Md.
IRL C. SCHOONOVER, 17, National Bureau of Standards, Washington, D. C.
ISAAC SCHOUR, 3, 808 S. Wood St., Chicago, Ill.
WARREN R. SCHRAM, 3, 311 E. Chicago Ave., Chicago, Ill.
JOSEPH SCHROFF, 1, 119 West 57th St., New York, N. Y.
FREDERICK W. SCHUBERT, 6, 1570 Eighth Ave., San Francisco, Calif.
ADOLPH SCHULTZ, 20, Medical School, Johns Hopkins U., Baltimore, Md.
EDWIN W. SCHULTZ, 6, Stanford U., Palo Alto, Calif.
E. O. SCOTT, Dental School, U. of Michigan, Ann Arbor, Mich.
HARRY M. SEMANS, 18, Dental School, Ohio State U., Columbus, Ohio.
WILLIAM H. SEBRELL, JR., 17, National Institute of Health, Bethesda, Md.
H. JOBE SEDWICK, Algronix Bldg., Washington and Spring Sts., Titusville, Pa. With U. S. Army.
BEN SEIDLER, 1, 57 West 57th St., New York, N. Y.
HARRY SHAPIRO, 1, 632 West 168th St., New York, N. Y.
JOHN S. SHELL, 3, Thos. Dee & Co., 1900 W. Kinzie St., Chicago, Ill.
HARRY SICHER, 2, Chicago Medical College, 710 S. Walcott St., Chicago, Ill.
ELI H. SIEGEL, 1, 630 West 168th St., New York, N. Y.
HANNAH E. SILBERSTEIN, 32, 1121 Michigan St., Indianapolis, Ind.
HERBERT F. SILVERS, 1, 7 West 96th St., New York, N. Y.
W. J. SIMON, 7, 4511 Wentworth Ave., Minneapolis, Minn.
NINA SIMMONDS, 6, Dental School, U. of California, San Francisco, Calif.
NORMAN F. SIMMONS, 1, 630 West 168th St., New York, N. Y.
F. V. SIMONTON, 450 Sutter St., San Francisco, Cal.
J. A. SINCLAIR, 201–212 Legal Bldg., Ashville, N. C.
WILLIAM G. SKILLEN, 3, 311 E. Chicago Ave., Chicago, Ill.
EUGENE W. SKINNER, 3, 311 E. Chicago Ave., Chicago, Ill.
ERNEST A. SLOMAN, 6, 344 Fourteenth St., San Francisco, Calif.
MARGARET C. SMITH, Agricultural Experiment Station, U. of Arizona, Tucson, Ariz.
REIDEV SOGNNAES, Medical-dental School, U. of Rochester, Rochester, N. Y.
RALPH F. SOMMER, 5, Dental School, U. of Michigan, Ann Arbor, Mich.

* Charter member.

SIDNEY SORRIN, 1, 745 Fifth Ave., New York, N. Y.
WILMER SOUDER, 17, National Bureau of Standards, Washington, D. C.
HARRY D. SPANGENBERG, JR., 18, 2861 Neil St., Apt. 461B, Columbus, Ohio.
BENJAMIN SPECTOR, 2, 60 The Fenway, Boston, Mass.
THOMAS D. SPEIDEL, 32, Dental School, U. of Indiana, Indianapolis, Ind.
EDWARD S. STAFNE, 7, Mayo Clinic, Rochester, Minn.
FREDERICK L. STANTON, 175 East 79th St., New York, N. Y.
S. R. STEADMAN, 7, 1201 Lowry Medical Arts Bldg., St. Paul, Minn.
GEORGE STEIN, Dental School, Harvard U., Boston, Mass.
M. RUSSELL STEIN, 1, 113 West 57th St., New York, N. Y.
ROBERT W. STEPHAN, 3, 25 E. Washington St., Chicago, Ill.
* PAUL R. STILLMAN, 1, Longwood, Florida.
EUGENE R. STONE, 17, 1726 Eye St., N. W. Washington, D. C.
ROY A. STOUT, 17, Walter Reed General Hospital, Washington, D. C.
WILLIAM F. SWANSON, 9, 5326 Pocusset St., Pittsburgh, Pa.
JAMES T. SWEENEY, 6, State Hospital, Stockton, Calif.
WILLIAM T. SWEENEY, 17, P. O. Box 1587, Pittsburgh, Pa.

LOREN B. TABER, 6, 291 Geary St., San Francisco, Calif.
MAURICE L. TAINTER, 6, 3563 Jackson St., San Francisco, Calif.
DAVID TANCHESTER, 1, 180 West 58th St., New York, N. Y.
EDWARD TAYLOR, State Dept. of Health, Austin, Texas.
NORRIS O. TAYLOR, 211 South 12th St., Philadelphia, Pa.
P. B. TAYLOR, 14, 1, 74th St., Brooklyn, N. Y.
RALPH W. TAYLOR, 17, U. S. Naval Dental School, Bethesda, Md.
WALTER E. TAYLOR, 9 St. Bernard Ave., Saranac Lake, N. Y.
BENJAMIN TENENBAUM, 1, 33 West 42nd St., New York, N. Y.
GEORGE W. TEUSCHER, 3, 311 E. Chicago Ave., Chicago, Ill.
* KURT H. THOMA, 2, Medical School, Harvard U., Boston, Mass.
B. O. A. THOMAS, 630 West 168th St., New York, N. Y.
NEWTON G. THOMAS, 3, 25 E. Washington St., Chicago, Ill.
HARRY S. THOMPSON, 4, 18 Summerhil Gardens, Toronto, Canada.
GEORGE E. THOMPSON, 2, Dental School, Harvard U., Boston, Mass.
J. R. THOMPSON, 3, 808 S. Wood St., Chicago, Ill.
A. H. THRONDSON, 6, 344 Fourteenth St., San Francisco, Calif.
SIDNEY TIBLIER, 29, 1141 Canal Bank Bldg., New Orleans, La.
BENJAMIN TISHLER, 358 Commonwealth Ave., Boston, Mass.
HARRY TRIMBLE, 2, 25 Shattuck St., Boston, Mass.
CHARLES TULLER, 29, 729 Maison Blanche, New Orleans, La.
CHARLES R. TURNER, 8, Dental School, U. of Pennsylvania, Philadelphia, Pa.
STANLEY D. TYLMAN, 3, 185 N. Wabash Ave., Chicago, Ill.

GRANT VAN HUYSEN, Dental School, U. of Indiana, Indianapolis, Ind.
L. E. VAN KIRK, 9, Jenkins Arcade, Pittsburgh, Pa.
FRANCIS B. VEDDER, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
WILLIAM D. VEHE, 7, 1001 Medical Arts Bldg., Minneapolis, Minn.
RAYMOND F. VINES, Research Lab., International Nickel Co., Bayonne, N. J.
J. F. VOLKER, 2, Dental School, Tufts College, Boston, Mass.
ROSCOE H. VOLLAND, 27, First National Bank Bldg., Iowa City, Iowa.
RALPH S. VOORHEES, 23, 311 Alexander St., Rochester, N. Y.
CHARLES J. VOSMIK, 14, 1338 Keith Bldg., Cleveland, Ohio.

* Charter member.

EDWARD C. WACH, 3, 3151 West 59th St., Chicago, Ill.
HAROLD F. WAHLQUIST, 7, 835 Medical Arts Bldg., Minneapolis, Minn.
WILLIAM W. WAINWRIGHT, 6, Dental School, U. of California, San Francisco, Calif.
FREDERICK C. WAITE, 14, 144 Locust St., Dover, New H.
CHARLES M. WALDO, 2, 25 Shattuck St., Boston, Mass.
CARL F. WALDRON, 7, Dental School, U. of Minnesota, Minneapolis, Minn.
DONALD A. WALLACE, 212 East Superior St., Chicago, Ill.
MARCUS L. WARD, 5, Dental School, U. of Michigan, Ann Arbor, Mich.
FRIEDRICH WASSERMAN, 3, 950 East 59th St., Chicago, Ill.
* LEUMAN M. WAUGH, 1, 931 Fifth Ave., New York, N. Y.
HAROLD L. WEATHERFORD, 2, 25 Shattuck St., Boston, Mass.
J. P. WEINMANN, 1757 West Harrison St., Chicago, Ill.
DAVID WEISBERGER, 2, Dental School, Harvard U., Boston, Mass.
W. H. WELKER, 3, 1853 W. Polk St., Chicago, Ill.
GEORGE D. WESSINGER, 3, 1757 W. Harrison St., Chicago, Ill.
LAURENCE G. WESSON, 2, 131 Park Drive, Boston, Mass.
RUSSELL C. WHEELER, 10, Missouri Theater Bldg., St. Louis, Mo.
R. C. WILLETT, 3, 535 Jefferson Bldg., Peoria, Ill.
JOHN B. WILLIAMS, 22, 1000 W. Grace St., Richmond, Va.
WARREN WILLMAN, 3, 1757 W. Harrison St., Chicago, Ill.
EDMUND M. WISE, Research Lab., International Nickel Co., Bayonne, N. J.
E. ALFRED WOLF, 9, Dept. of Zoology, U. of Pittsburgh, Pittsburgh, Pa.
FREDERICK J. WOLFE, 29, 835 Maison Blanche, New Orleans, La.
HAROLD G. WORMAN, 7, 508 LaSalle Bldg., Minneapolis, Minn.
WALTER H. WRIGHT, 9, Dental School, U. of Pittsburgh, Pittsburgh, Pa.
W. L. WYLIE, 14, 2165 Adelbert Road, Cleveland, Ohio.

MORTON F. YATES, 2, Dental School, Harvard U., Boston, Mass.
E. GORDON YOUNG, 11, Dalhousie U., Halifax, Nova Scotia.

H. A. ZANDER, 2, Dental School, Tufts College, Boston, Mass.
EARL A. ZAUS, 3, 311 E. Chicago Ave., Chicago, Ill.
ROBERT C. ZEISZ, 6, 490 Post St., San Francisco, Calif.
JAMES L. ZEMSKY, 1, 147 Fourth Ave., New York, N. Y.
DANIEL E. ZISKIN, 1, 630 West 168th St., New York, N. Y.

Total number of North American members—488.

2. MEMBERSHIP ROLL OF COLLEAGUES RESIDENT OUTSIDE OF NORTH AMERICA LISTED BY SECTIONS

Budapest Section.—Károly Balogh, István Bánhegyi, Béla Bonyhárd, János Brand, Béla de Simon, Károly Ferenczy, Dezsö Hattysazy, Oszkár Kömives, **Ferenc Kőszeg*, Ervin Landgraf, Guztáv Morelli, Rudolph Rehák, Henrik Salamon, László Sugár, Steven Varga, Dénes von Máthé.—16.

Chengtu Section.—Mary C. Agnew (Mrs. R. G.), **R. G. Agnew*, H. B. Collier, L. G. Kilborn, A. W. Lindsay, H. J. Mullet.—6.

Johannesburg Section.—G. Friel, J. N. Noriskin, T. W. Osborn, J. C. M. Shaw, Julius Staz, **W. A. Wilson.*—6.

London Section.—Arthur Bulleid, Sir J. F. Colyer, E. B. Dowsett, E. W. Fish, C. W. Howkins, G. B. Pritchard, M. A. Rushton, **Evelyn Sprawson*, Joseph Turner.—9.

Palestine Section.—Heinrich Berger, Hugo Brasch, Ernst Kellner, **Samuel Lewin-Epstein*, Ervin Mansbach, Abraham Sussman, Jacob Yardeni.—7.

* Charter member.

Prague Section.—Karel Černy, Karl Häupl, Ján Jesenský, František Kostečka, Jaromir Křečan, A. E. Loos, F. Náprstek, **Friedrich Neumann*, František Neuwirth, Čestimir Parma, Josef Přibyl, Ferdinand Škaloud, Karel Wachsmann, Hans Wermuth.—14.

Vienna Section.—Arwed Berg, **Fritz Driak*, Richard Grohs. Otto Hofer, Hermann Mathis, Hans Pichler, Otto Preissecker, Franz Schönbauer, A. M. Schwartz, Karl Spring, Richard Trauner, Hermann Wolf.—12.

Not residents of North America and not now members of a Section—J. L. Boots, Ove Brinch, Gyula Csernyei, C. D. M. Day, Jorge Erausquin, Guido Fischer, S. F. Gottlich, Walter Hess, R. E. T. Hewat, J. J. Holst, G. Y. Hildebrand, Frode Hilming, Humphrey F. Humphreys, J. T. Irving, T. E. de Jonge-Cohen, Gustav Korkhaus, G. D. Macphee, Maurine W. Müller, Pekka Nironen, Silvio Palazzi, P. O. Pedersen, Paul Pincus, Friedrich Proell, F. M. Pucci, Inghold Reichborn, Elkan Sanders, Guttrom Toverud, Rudolph Weber, J. J. Wolfe, H. K. Worner—30.

Total number of members as of Dec. 1, 1942—588.

BOOKS RECEIVED, 1942

Bernier, J. L., Mann, J. B., and Ash, J. E., *Atlas of Dental and Oral Pathology*. 2nd Ed. (Chicago, American Dental Association: 1942), pp. 178+.

Bernier, J. L., *Differential Diagnosis of Oral Lesions*. (St. Louis, C. V. Mosby: 1942), pp. 228.

Council on Dental Education, *Proceedings of the Second Congress on Dental Education and Licensure*. (American Dental Association, Chicago: 1942), pp. 76.

Darlington, C. G., Wilson, G. W., Miller, H. C., Wright, W. H. and Moore, G. R., *The 1941 Year Book of Dentistry*. (Chicago: Year Book Publishers: 1940), pp. 792.

Dewey, M., and Anderson, G. M., *Practical Orthodontics*. 6th Ed. (St. Louis, C. V. Mosby: 1942), pp. 545.

Hoskins, M. M., and Bevelander, G., *Outline of Histology*. (St. Louis, C. V. Mosby: 1942), pp. 112.

Miller, R. G., *Synopsis of Full and Partial Dentures*. (St. Louis, C. V. Mosby, 1942), pp. 221.

Moulton, F. R. (editor), *Fluorine and Dental Health*. Publication of the American Association for the Advancement of Science No. 19. (Washington, Am. Assoc. Advancm. Science: 1942.)

Wesson, L. G., *Chemistry of Dental Materials*. (St. Louis, C. V. Mosby: 1942), pp. 106.

INDEX

Volume 21, 1942[1]

ABRASION: dentifrice, 335*
——: gold alloys, 103
Acid: production; bacteria, 61, 461
Acid-base: balance; caries, 338*, 437
Acidogens: oral, 263
Age: radiophosphorus metabolism, 339*
——: tooth; caries rate, 135
Ageing: dentin, 302*, 423
AISENBERG, M. S.: epithelium; pulp, 326*
Alloy: chrome; implants, 306*
——: electrographic analysis, 306*
Alveoloclasia: rate, 241*, 311*, 365
Amalgam: contamination, 302*
——: expansion, 307*
——: expansion; zinc, 307*
Ameloblastoma: incidence and histology, 529
——: microincineration, 297*
Anodontia: cephalometric study, 329*
Antibody: regional lymph node, 309*
Antiseptic: dentin, 115
Antiseptics: effectiveness; cavity preparation, 269
Aphtha: recurrent oral, 335*
APPLEBAUM, E.: enamel; shark, 251
——: epithelial sheath, 331*, 403
APPLETON, J. L. T.: bacteria; sneezing, 309*
——: saliva: capillary permeability, 505
ARMSTRONG, W. D.: diet; teeth and jaws, 335*, 513
——: fluorine; diet, 326*
——: radiocalcium; bones and teeth, 326*
ARNIM, S. S.: statistics; diagnosis, 243
ARNOLD, F. A., JR.: fluorine; caries rate, 303*
——: ——; lactobacillus count, 304*

BACTERIA: acidogenic properties, 61, 241*, 263, 321*, 461
——: anaerobes, 5, 543
——: filamentous; stained teeth, 326*
——: lactic and pyruvic acids, 241*, 321*
Bacteria, (*con.*):
——: nutritional requirements, 259, 310*, 467
——: oral mucosa; effect of drying, 304*, 379
——: post-mortem studies; teeth, 9
——: saliva; acid; caries, 328*
——: sneezing, 309*
See also under generic names.
BARNUM, C. P.: radiocalcium; bones and teeth, 326*
BARTELS, H. A.: filamentous organism; stained teeth, 326*
——: sterilization; bacteriological tests, 308*
BAUER, W. H.: syphilis; jaws and teeth, 299*
BEALL, J. R.: amalgam; contamination, 302*
BECKS, H.: salivary calcium and phosphorus; flavored activators, 87
BENZER, S.: caries; secondary dentin, 318*
BERGER, A.: central giant cell tumor, 327*
BERNICK, S.: triplets; arch form and pattern, 413
——: ——; arch width and length, 233
BERNIER, J. L.: ameloblastoma, 529
——: vitallium implants; reaction, 327*
BEUBE, F. E.: bone powder; periodontium, 298*
BEVELANDER, G.: caries; secondary dentin, 318*
——: tooth fracture; reaction, 327*, 481
BIBBY, B. G.: bacteria; acid production; decalcification, 61
——: caries; fluorine, 314*
——: saliva; acid; bacteria; caries, 328*
BIRD, M. J.: composition; enamel; dentin, 323*
BLATTNER, R. J.: osteogenesis imperfecta; odontogenesis imperfecta, 325*
Blood: examination; extraction, 19
——: periodontoclasia, 310*
——: vitamin C level, 330*, 353

[1] Abbreviations used: I. A. D. R.—International Association for Dental Research; J. D. R.—Journal of Dental Research.
* Abstract.

Bodecker, C. F.: dental caries; acid-base balance, 338*, 437
——: dental papilla; tooth formation, 301*, 391
——: inaugural address, 291, 326*
——: introduction, 289, 326*
Bone: alveolar; forces, 241*
——: disease; caries; growth, 336*
——: ——; dentin formation, 325*
——: formation; epithelium, 298*
——: histology; periodontal disease, 35
——: pathology; 330*
——: powder; periodontium, 298*
——: sodium absorption, 323*
Bonner, J. F.: calcium phosphates; x-ray diffraction study, 324*
——: sodium metabolism; dental tissues, 323*
Books received, 578
Borden, I.: pulp treatment, 329*
Bradley, J. L.: pulp; inflammation; symptoms, 334*
Breitner, C.: alveolar bone; forces, 241*
——: occlusal changes; masticatory muscles, 300*
Brodie, A. G.: anodontia; cephalometric study, 329*
——: mandible; position and form, 339*
Brodsky, R. H.: caries; vitamin D, 314*
——: leukoplakia; grenz ray, 329*
Burket, L. W.: dentin microscopy, 299*
——: post-mortem bacteriologic studies, 9
Burrill, D. Y.: silver nitrate penetration, 319*
——: vitamin C; blood; caries, 330*
——: vitamin C; gingival and periodontal disease, 330*, 353

Cadmium: dental effects, 302*
Cahn, L. R.: bone formation; epithelium, 298*
——: bone pathology, 330*
——: cysts; fat, 330*
——: mast cell, 331*
——: pseudoepitheliomatous hyperplasia, 331*
Calamari, J. A.: alloys; electrographic analysis, 306*
Calcium: bones and teeth, 35*, 513
——: radioactive; bones and teeth, 326*
——: saliva; caries, 83, 87
Calcium phosphate: x-ray diffraction study, 324*
Campbell, J.: pulp treatment, 329*
Canby, C. P.: vitallium implants; reaction, 327*
Cancer: mandible, 325*
Capillary: permeability; saliva, 505
Caries: acid-base balance, 338*, 437
——: bacteria. *See under* bacteria and generic names.
——: Bergen County, N. J., 125
——: bone diseases, 336*
——: contact surfaces, 318*, 473
——: 358 counties, 332*, 455
——: diagnosis, 243
——: economic status, 320*
——: hydrogen ion modification; agents, 315*
——: fluorine, 139, 303*, 305*, 314*, 320*
——: incidence; Indiana Univ. students, 312*
——: masticatory stresses, 319*, 387
——: Panama Canal Zone, 55
——: pathology, 317*
——: rat; mechanical factors, 333*
——: rate, 333*, 443
——: rate; tooth age, 135
——: resistance, 317*, 475
——: saliva; acid; bacteria, 328*
——: ——; calcium; phosphorus, 83
——: ——; opsonin, 333*, 509
——: ——; pH, 73
——: secondary dentin, 318*
——: streptococcus, 318*
——: tuberculosis, 312*
——: vital and pulpless teeth, 332*
——: vitamin B, 259
——: vitamin C, 330*
——: vitamin D, 314*
Chapin, R. W.: caries; Panama Canal Zone, 55
Cheilosis: angular; vertical dimension increase, 305*
Chemotherapy: see specific drugs
Cheyne, V. D.: caries; fluorine, 320*
——: ——; Indiana University students, 312*
——: fluorine; mottling, 145
Cohen, J. T.: triplets; arch form and pattern, 413
——: ——; arch width and length, 233
Colchicine: dental growth, 316*
Composition: dentin; enamel, 323*
——: enamel; histology, 171, 323*, 429
Contact surfaces: caries, 318*, 473

* Abstract.

Cooper, E. N.: hardness; synthetic resin, 307*
Criswell, J. C.: caries; fluorine, 305*
Cyst: fat, 330*

Dale, P.: cadmium; dental effects, 302*
Deakin, M.: developing enamel; chemistry, 321*, 429
Dean, H. T.: fluorine; caries rate, 303*
——: ——; latobacillus count, 304*
Decalcification: bacteria, 61
De Ford, C. C.: prostatic restorations; navy personnel, 493
Dentifrice: abrasion, 335*
Dentin: abrasion; dentifrice, 335*
——: composition, 323*
——: growth; strontium, 497
——: hardness; drying, 99
——: hereditary opalescent, 325*
——: ——; roentgen-ray absorption, 107
——: metamorphosis, 302*, 423
——: ——; pain, 338*
——: microscopy, 299*
——: opalescent; retinitis pigmentosa, 337*
——: secondary; caries, 318*
——: sodium absorption, 323*
——: solution; inorganic acids, 322*
Deposit: organic structureless; teeth, 322*
Desauer, G.: sodium metabolism; dental tissues, 323*
Detergents: hydrogen ion modification, 315*
Dextrose: lactobacilli, 310*
Diabetes: teeth and gingiva, 296*
Diagnosis: pulpitis, 334*
——: statistics, 243
Diamond, M.: epithelial sheath, 331*, 403
Diet: bones and teeth, 335*, 513
——: fluorine, 139, 157, 326*
See also under nutrition, calcium, fluorine, vitamin, etc.
Dietz, A. K.: bacteria; sneezing, 309*

East, B. R.: caries; Bergen County, N. J., 125
——: ——; 358 counties, 332*, 455
——: caries rate; tooth age, 135
Economic status: caries, 320*
Ehrich, W. E.: lymph node; antibody formation, 309*
Elvove, E.: fluorine; caries rate, 303*
——: ——; lactobacillus count, 304*
Enamel: chemical and histologic study, 171, 321* 429
Enamel, (*con.*):
——: composition, 323*
——: development, 183
——: hereditary amelogenesis imperfecta, 306*
——: shark, 251
——: sodium absorption, 323*
Epithelial sheath, 331*, 405
Epithelium: bone formation, 298*
——: pulp, 326*
Eruption: continuously growing teeth, 201
——: teeth of limited growth, 395
Extraction: blood examination, 19

Failla, P. S.: blood examinations; extraction, 19
Fat: cyst, 330*
Feldman, B. L.: caries; vital and pulpless teeth, 332*
Fleeman, J.: osteology; guinea pig skull, 333*
Florestano, H. J.: bacteria; acidogenic properties, 263
Fluorine: caries, 139, 303, 305*, 314*, 320*
——: diet, 157, 326*
——: salivary lactobacillus, 304*
Foley, G.: fusospirochetes; infectivity, 309*, 375
——: spirochetes; cultivation, 242*, 308*
Foster, W. C.: caries; dietary fluorine, 139, 305*
Fracture: tooth; experimental, 327*, 481
Freeman, S.: brown opalescent dentin; retinitis pigmentosa, 337*
Furuta, W. J.: fluorine; dry and moist diets, 157
Fusiform organisms: edentulous mouth, 308*, 373
——: infectivity, 309*, 375

Gafafer, W. M.: caries; rate, 333*, 443
Gallup, H.: composition; enamel; dentin, 323*
Gaudino, J.: composition; enamel; dentin, 323*
Gies, William J.: 1
Gilda, J. E.: metabolism; pulpless teeth, 322*
Gingiva: diabetes, 296*
——: hypothyroidism, 296*
Gingival crevice: alveolar crest, 561
Gingivitis: alveolar crest relationship, 561

* Abstract.

Gingivitis, (*con.*):
——: chronic desquamative; sex hormones, 341*
——: oxygen 313*
——: vitamin C, 330*, 353
GINN, J. T.: cadmium; dental effects, 302*
——: caries; rate; mechanical factors, 333*
——: radiophosphorus metabolism; teeth, 339*
——: ——; pulpless teeth, 322*
——: salivary gland extirpation; rat, 297*
——: sodium metabolism; dental tissues, 323*
——: tooth movement; molar loss, 301*
GLICKMAN, I.: Paget's disease; jaws, 300*
——: periodontal disease; bone histology, 35
GLOCK, G. E.: enamel development; dogs, 183
GLYCOGEN: fetal heads, 519
Gold: alloys; abrasion, 103
GOLDMAN, H. M.: alveolar crest and gingival crevice relationship, 561
GOOTZEIT, E. H.: fusospirochetal organisms; edentulous mouth, 308*, 373
GORDON, S. M.: dental science and dental art grant-in-aid, 326*
GORE, J. T.: saliva; mucin, 299*, 383
GOTTLIEB, B.: caries; pathology, 317*
Grenz ray: leukoplakia, 329*
GRIESS, F.: sulfanilamide; local, 3
GROSSMAN, L. I.: dentin; solution; inorganic acids, 322*
Growth: bone diseases, 336*
——: colchicine study, 316*
——: dental papilla, 301*, 391
——: dentin; strontium, 497
——: enamel; chemistry, 171, 321*, 429
——: glycogen; fetal heads, 519
——: hibernation, 336*
——: phosphatase; fetal heads, 519
GRUBER, I. E.: tuberculosis; teeth and periodontium, 312*
Guinea pig: skull, 333*
Gynecological disorders: oral and vaginal smears, 341*

HAMMOND, C. W.: saliva; opsonin, 279
——: ——; ——; caries, 333*, 509
HAMMOND, W. M.: dentin microscopy, 299*
HAMPP, E. G.: ameloblastoma; microincineration, 297*
Hardness: dentin; drying, 99
——: Knoop indentation instrument, 303*
——: synthetic resin, 307*
HARRIS, T. N.: lymph node; antibody formation, 309*
HARRISON, R. W.: lactobacillus; sensitization of rabbits, 223
HATTON, E. H.: I. A. D. R.; members, 566
——: pulp; inflammation; symptoms, 334*
——: salivary acidophilus; paraffin and gum, 334*
HEALEY, H. J.: caries; Indiana University students, 312*
HEMPHILL, C. D.: mucosal bacterial count; drying, 304*, 379
Heredity: amelogenesis imperfecta, 306*
——: opalescent dentin; osteogenesis imperfecta, 325*
——: ——; roentgen-ray absorption, 107
Hertwig: sheath, 331*, 405
HEYS, F.: osteogenesis imperfecta; odontogenesis imperfecta, 325*
Hibernation: tooth development, 336*
HILL, T. J.: lactobacilli; pantothenic acid and dextrose, 310*, 467
HILLIER, J.: electronic microscope, 326*
HINDS, E.: caries; pathology, 317*
——: ——; resistance, 317*, 475
HINDS, F. W.: caries; contact surfaces, 318*, 473
HODGE, H. C.: composition; enamel; dentin, 323*
——: sodium metabolism; dental tissues, 232*
HOFFMAN, M. M.: fluorine; dry and moist diets,157
HOOK, W. E.: hibernation; tooth development, 336*
Hormones: sex; gingivitis, 341*
HOROWITZ, N. H.: glycogen and phosphatase; fetal heads, 519
Hydroxylapatite: sodium absorption, 323*
Hyperplasia: pseudoepitheliomatous, 331*
Hypersensitivity: lactobacillus, 316*

I. A. D. R.: members, 566
——: New York Section; proceedings, 241, 421
——: proceedings; address; inaugural, 291, 326*
——: ——; ——; presidential, 285, 326*
——: ——; contents, 285

* Abstract.

I. A. D. R. (*con.*):
——: ——; executive, 344
——: ——; index of participants, 295
——: ——; introduction of president-elect, 289, 326*
——: ——; J. D. R., 346
——: ——; members; new, 349
——: ——; necrology, 342
——: ——; officers, 349
——: ——; reports; officers and committees, 344
——: work and functions, 285, 326*
Implantation: chrome alloys, 306*
Indians: prehistoric; dental conditions, 211
Infection: focal; post-mortem studies, 9
Investment: vulcanizing; strength of plaster, 340*
Isaacs, A.: necrology, 342

J. D. R., 2, 346
——: sustaining subscribers, 565
Jump, E. B.: colchicine; dental growth, 316*

KAMEN, P.: gynecological disorders; oral and vaginal smears, 341*
Karshan, M.: caries; saliva; calcium; phosphorus, 83
——: periodontoclasia; blood studies, 310*
——: ——; salivary studies, 311*
Kelly, V.: radiocalcium; bones and teeth, 326*
Keratinization: oral and vaginal mucosa, 341*
Kittay, I.: gynecological disorders; oral and vaginal smears, 341*
Klatsky, M.: caries; masticatory stresses, 319*, 387
Klein, H.: caries; economic status, 320*
Kniesner, A. H.: caries; vitamin B deficiency, 259
——: lactobacilli; pantothenic acid and dextrose, 310*, 467
Knoop: indentation hardness instrument, 303*
Knowlton, M.: fluorine; diet, 326*
Kogan, F.: periodontal membrane; width; young individual, 337*
Koss, W. F.: sodium metabolism; dental tissues, 323*
Kotanyi, E.: cancer; mandible, 325*
——: thrombocytopenic purpura, 334*
Krasnow, F.: I. A. D. R., New York Section; Proceedings, 241, 421
Kreshover, S.: dentin microscopy, 299*
——: tuberculosis; teeth; mice, 27

LACTOBACILLUS: fluorine, 304*
——: hypersensitivity, 316*
——: pantothenic acid and dextrose, 310*, 467
——: paraffin and gum chewing, 334*
——: sensitization of rabbits, 223
Lasater, R. L.: gold alloys; abrasion, 103
Lefkowitz, W.: caries; vital and pulpless teeth, 332*
——: dental papilla; tooth formation, 301*, 391
——: dentin; metamorphosis, 302*, 423
Leukoplakia: grenz ray, 329*
Levy, B. M.: capillary permeability; saliva, 505
Liebig, E. O.: amalgam; zinc; expansion, 307*
Ludwick, N. V.: caries; fluorine, 305*
Lund, A. P.: diet; teeth and jaws, 335*, 513
Lymph node: antibody, 309*

MANDIBLE: form and position, 339*
Manly, R. S.: dentin abrasion; dentifrice, 335*
——: structureless organic deposit; teeth, 322*
Mann, A. W.: angular cheilosis; vertical dimension increase, 305*
——: caries; vitamin B deficiency, 259
Marshall, J. A.: necrology, 342*
Mast cell, 331*
Mastication: stress; caries, 319*, 387
Maurer, D. W.: saliva; acid; bacteria; caries, 328*
McCauley, H. B.: hereditary opalescent dentin; roentgen-ray absorption, 107
——: radiophosphorus metabolism; whole teeth, 323*
McClendon, J. F.: caries; dietary fluorine, 139
——: ——; fluorine, 305*
McDonagh, A. J.: necrology, 343
Mellanby, H.: enamel development; dogs, 183
Mellanby, M.: enamel development; dogs, 183
Metabolism: teeth, 322*, 323*, 326*, 339, 423

* Abstract.

Microincineration: ameloblastoma, 297*
Microscope: electronic, 326*
MILLER, B. F.: hydrogen ion modification; agents, 315*
MILLER, H. G.: dentin abrasion; dentifrice, 335*
MILLER, S. C.: alveoloclastic experience; teeth, 241*, 311*, 365
MILLS, C. A.: caries; Panama Canal Zone, 55
MORSE, F. W., JR.: root canal; anaerobic cultures, 5
Mottled enamel: fluorine, 145
Mucin: saliva, 299*, 383
Mucosa: oral; hypothyroidism, 296*
MUNTZ, J. A.: detergents and urea; influencing factors, 315*
MURRAY, M. M.: enamel development; dogs, 183
Muscles: masticatory; occlusal changes, 300*

NAVY personnel; prosthetic restorations, 493
Necrology: I. A. D. R., 342
Neoplasm: ameloblastoma, 297*
——: cancer; mandible, 325*
——: central giant cell, 327*
——: pseudoepitheliomatous hyperplasia, 331*
NEUWIRTH, I.: bacteria; lactic and pyruvic acids, 241*, 321*
NUCKELLS, J.: pulp treatment, 329*
Nutrition: see also vitamins and mineral elements.

OCCLUSAL changes: masticatory muscles, 300*
Odontogenesis imperfecta: osteogenesis imperfecta, 325*
——: See hereditary opalescent dentin.
OLIVER, C. P.: triplets; arch form and pattern, 413
——: ——; arch width and length, 233
OMAN, C. R.: pulp capping, 296*
Opsonin: saliva, 279
——: ——; caries, 333*, 509
ORBAN, B.: oxygen; gingival pocket, 313*
Orthodontics: alveolar bone; forces, 241*
——: arch width and length; triplets, 233
——: first molar lost; position of second premolar, 300*, 489
Orthodontics, (*con.*):
——: occlusal changes; masticatory muscles, 300*
——: second molar lost; movement of first and third molars, 301*
Osteogenesis imperfecta: odontogenesis imperfecta, 325*
Osteology: guinea pig skull, 333*
Oxygen: gingival pocket, 313*

PAGET'S disease: jaws, 300*
Pain: dentin metamorphosis, 338*
Panama Canal Zone: caries, 55
Pantothenic acid: lactobacilli, 310*
Papilla: dental; tooth formation, 301*, 391
Periodontal disease: bone histology, 35
——: gingival crevice relation, 561
——: tuberculosis, 312*
——: vitamin C, 330*, 353
Periodontal membrane: width; young individual, 337*
Periodontics: acrylic splints, 337*
——: alveoloclastic rate, 241*, 311*, 365
Periodontium: bone powder, 298*
Periodontoclasia: blood studies, 310*
——: salivary studies, 311*
pH: saliva; caries, 73
Phosphatase: fetal heads, 519
Phosphorus: saliva; caries, 83, 87
Plaster: strength as vulcanizing investment, 340*
POHLEN, K.: caries; Bergen County, N. J., 125
——: ——; 358 counties, 332*, 455
POWELL, V. H.: second molar lost; first and third molar movement; rat, 301*
Prosthetic restorations: Navy personnel, 493
Pulp: capping, 296*
——: epithelium, 326*
——: inflammation; symptoms, 334*
——: treatment, 329*
Pulpless teeth; metabolism, 322*
Purpura: thrombocytopenic, 334*

RABKIN, S.: dental conditions; prehistoric Indians; Alabama, 211
——: sound conduction; dental operation, 313*
Radioactive substances; saliva, 324*
Radioautographs: 323*

* Abstract.

Radiocalcium: bones and teeth, 326*
Radiograph. *See under* X-ray.
Radiophosphorus: metabolism; age and species, 339*
——: ——; pulpless teeth, 322*
——: ——; teeth, 323*, 339*
Radiosodium: metabolism; teeth, 323*
RAY, K. W.: amalgam; expansion, 307*
REED, G.: enamel, chemical and histologic study, 171
Resin: synthetic; hardness, 307*
Retinitis pigmentosa: brown opalescent dentin, 337*
RICE, E.: sterilization; bacteriological tests, 308*
RICE, T. B.: caries; fluorine, 320*
ROBINSON, H. B. G.; Gies, W. J., 1
——: I. A. D. R.; proceedings, 285
——: osteogenesis imperfecta; odontogenesis imperfecta, 325*
——: this index, 579
Roentgen-ray. *See under* x-ray
Root canal: anaerobic cultures, 5
ROSEBURY, T.: fusospirochetes; infectivity, 309*, 375
——: spirochetes; cultivation, 242*, 308*
ROSENSTEIN, S. N.: recurrent oral aphthae, 335*
ROSENTHAL, S. L.: fusospirochetal organisms; edentulous mouth, 308*, 373

SALIVA: acid; bacteria; caries, 328*
——: calcium, 87
——: capillary permeability, 505
——: caries; calcium; phosphorus, 83
——: ——; opsonin, 333*, 509
——: lactobacillus count, 304*
——: mucin, 299*, 383
——: opsonin, 279, 333*, 509
——: periodontoclasia, 311*
——: pH; caries, 73
——: phosphorus, 87
——: radioactive substances, 324*
——: secretion; cat, 324*
Salivary gland: extirpation; rat, 297*
SALZMANN, J. A.: first molar lost; position of second premolar, 300*, 489
SARNAT, B. G.: anodontia; cephalometric study, 329*
——: hibernation; tooth development, 336*
SCHICK, B.: caries; vitamin D, 314*
SCHLACK, C. A.: mucosal bacterial count; drying, 304*, 379
SCHLACK, C. A. (*con.*):
——: prosthetic restorations; Navy personnel, 493
SCHOONOVER, I. C.: amalgam; contamination, 302*
SCHOUR, I.: I. A. D. R.; presidential address, 285, 326*
SCHUCK, C.: fluorine; dry and moist diets, 157
SEIDLER, B. B.: alveoloclastic experience; teeth, 241*, 311*, 365
SELTZER, S.: dentin sterilization; agents, 115
——: sterilizing agents; effectiveness, 269
Sensitization: lactobacillus, 223
SHAPIRO, H. H.: dental papilla; tooth formation, 301*, 391
Shark: enamel, 251
SICHER, H.: tooth eruption; continuously growing teeth, 201
——: ——; teeth of limited growth, 395
SIEGEL, E. H.: bone disease; caries; tooth growth, 336*
——: diabetes; teeth and gingiva, 296*
Silver nitrate: caries, 317*
——: penetration, 319*
SIMMONS, N. S.: salivary secretion; cat, 324*
SKILLEN, W. G.: brown opalescent dentin; retinitis pigmentosa, 337*
——: periodontal membrane; width; young individual, 337*
SKINNER, E. W.: gold alloys; abrasion, 103
——: hardness; synthetic resin, 307*
Sneezing: bacteria, 309*
SNYDER, M. L.: acid production; oral bacteria, 461
Sodium: metabolism; dental tissues, 323*
SORRIN, S.: acrylic splints; periodontal practice, 337*
SOUDER, W.: amalgam; contamination, 302*
Sound: dental operation, 313*
Species: radiophosphorus metabolism, 339*
SPIES, T. D.: angular cheilosis; vertical dimension increase, 305*
——: caries; vitamin B deficiency, 259
Spirochete: cultivation, 308*, 543
——: edentulous mouth, 308*, 373
——: infectivity, 309*, 375
Spints: acrylic; periodontics, 337*
SPRINGER, F. M.: angular cheilosis; vertical dimension increase, 305*
Statistics: caries; rate, 333*, 443
——: diagnosis, 243

* Abstract.

STEIN, G.: hypothryoidism; gingiva and oral mucosa, 296*
STEPHAN, R. M.: hydrogen ion modification; agents, 315*
Sterilization: bacteriological tests, 308*
——: dentin; agents, 115, 269
STOWE, L. R.: bone disease; caries; tooth growth, 336*
Streptococcus: caries, 318*
STROCK, A. E.: chrome alloys; implantation, 306*
Strontium: dentin opposition, 497
Sulfanilamide: postextraction use, 3
SUMMERSON, W. H.: bacteria; lactic and pyruvic acids, 241*, 321*
SVOBODA, J. F.: hereditary amelogenesis imperfecta, 306*
SWEENEY, W. T.: Knoop indentation hardness instrument, 303*
SWERDLOVE, C. K.: caries; salivary pH, 73
Syphilis: jaws and teeth, 299*

TAYLOR, D. N.: mucosal bacterial count; drying, 304*, 379
TEACHOUT, J. J.: acid production; oral bacteria, 461
TEFFT, H. I.: caries; streptococcus, 318*
TENNANBAUM, B.: periodontoclasia; blood studies, 310*
——: ——; salivary studies, 311*
THEWLIS, J.: enamel development; dogs, 183
THOMAS, B. O. A.: dental caries; acid-base balance, 338*, 437
——: dentin metamorphosis; pain, 338*
THOMPSON, J. R.: mandible; position and form, 339*
Thyroid: hypo; gingiva and oral mucosa, 296*
Tooth loss: orthodontic anomalies, 300*, 301*, 489
TOTAH, V. P.: dentin; hardness; drying, 99
Triplets: arch form and pattern, 413
——: arch width and length, 233
Tuberculosis: teeth, 27, 312*
Tumor. *See under* Neoplasm

UREA: hydrogen ion modification, 315*

VAN KESTEREN, M.: bacteria; acid production; decalcification, 61
Vitallium: implants; reaction, 327*
Vitamin B: deficiency; caries, 259
——: caries, 330*
Vitamin C: gingivitis; periodontal disease, 330*, 353
Vitamin D: bones and teeth, 335*, 513
——: caries, 314*
VOLKER, J. F.: bacteria; acid production; decalcification, 61
VOLKER, J. F. (*con.*): cadmium; dental effects, 302*
——: metabolism; pulpless teeth, 322*
——: radiophosphorus metabolism; age and species, 339*
——: ——; teeth, 339*
VOLLMER, H.: caries; vitamin D, 314*

WAINWRIGHT, W. W.: salivary calcium and phosphorus; flavored activators, 87
WAUGH, L. M.: I. A. D. R.; introduction of president-elect, 289, 326*
WEINMANN, J. P.: enamel; chemical and histologic study, 171
——: hereditary emelogenesis imperfecta, 306*
——: saliva; opsonin, 279
——: ——; ——; caries, 333*, 509
——: strontium; dentin growth, 497
WESSINGER, G. D.: enamel; chemical and histologic study, 171
WHITE, J. D.: necrology, 344
WICHELHAUSEN, O. W.: spirochetes; cultivation, 543
WICHELHAUSEN, R. H.: spirochetes; cultivation, 543
WILLIAMS, J.: radiocalcium; bones and teeth, 326*
WILLIAMS, N. B.: lactobacillus; hypersensitivity, 316*
——: lactobacillus; sensitization of rabbits, 223
WILLS, J. H.: saliva; radioactive substance, 324*
WOOD, H.: periodontal disease; bone histology, 35
WORNER, H. K.: dental plasters; strength as vulcanizing investments, 340*

X-RAY: absorption; hereditary opalescent dentin, 107
X-ray diffraction study: calcium phosphate, 324*

YATES, M. F.: root canal; anaerobic cultures, 5

ZANDER, H. A.: silver nitrate penetration, 319*
ZEGARELLI, E. V.: pulp capping, 296*
ZISKIN, D. E.: bone disease; caries; tooth growth, 336*
——: chronic desquamative gingivitis; sex hormones, 341*
——: diabetes; teeth and gingiva, 296*
——: gynecological disorders; oral and vaginal smears, 341*
——: hypothyroidism; gingiva and oral mucosa, 296*
——: recurrent oral aphthae, 335*

* Abstract.

BUY WAR BONDS AND STAMPS

It is our duty and our privilege to invest in America through the War Savings Program. Our purchase of War Bonds helps to buy the Nation's planes, tanks and guns. The money so invested reduces consumers' purchasing power and thereby acts as a curb upon inflation. And for the individual, the continued and systematic buying of Bonds and Stamps builds toward personal security for the future.